DEVELOPMENT, PRESERVATION AND MICROBIOLOGICAL QUALITY EVALUATION OF HURDLE PROCESSED BUFFALO MEAT PRODUCTS UNDER AMBIENT CONDITIONS

ABSTRACT

THESIS SUBMITTED FOR THE AWARD OF THE DEGREE OF

DOCTOR OF PHILOSOPHY

IN

AGRICULTURAL MICROBIOLOGY

By

MAHJABEEN SIDDIQUI

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F/O AGRICULTURAL SCIENCES
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A thesis submitted in fulfillment of the requirements for the degree of Doctor of Philosophy in Agricultural Microbiology of the Aligarh Muslim University, Aligarh.

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ABSTRACT

India is a major producer of buffalo meat with a total out put of about 1.4 million tones, owing to large population (~ 494.4 millions) of this animal. The production of buffalo meat in India contributes to about 50 % of world production and roughly, 85 % of it is exported from India. However, the meat in this country is mostly produced from aged and unproductive animals and, therefore, it is usually fibrous and tough in texture. Grinding of this kind of meat leads to rapid formation of metmyoglobin with undesirable brown colour and oxidative rancidity, which severely affects the consumer acceptance.

Since, raw meat is highly perishable in nature, it demands immediate processing and preservation. Meat processed through common techniques as freeze dehydration or thermal processing is not only expensive but also adversely affects the quality of meat. These major limitations could be easily surmounted with the simple and inexpensive approach of hurdle technology, which relies on optimal combination of several preservation factors or hurdles. So far, more than 60 potential hurdles for food, which improves the stability and /or quality of products have been reported. In industrialized countries, the hurdle technology approach is currently of much interest for minimally processed food, which are mildly heated or fermented, and for underpinning the microbial stability and safety of foods coming from healthful foods requiring minimal packaging. In developing countries, the application of hurdle technology for foods that remain stable, safe and tasty if stored without refrigeration is of paramount importance. Much interest in intentional hurdle technology is also emerging for meat product in China and for dairy products in India.

For each stable and safe food, a certain set of hurdles is inherent, which differs in quality and intensity depending on particular product. In any case, the hurdles must

keep the normal population of microorganisms in food under control. The initial population of microorganisms present in meat should not be able to leap over the hurdles present during storage of meat; otherwise, the meat will spoil and even cause food poisoning

The multi-target approach of food preservation involving the additive and/or synergistic effects of hurdles has prompted this study on development of two different hurdle-processed buffalo meat products namely pickles and powder. The study involves the effects of spices, condiments, acetic acid, sodium nitrite and potassium sorbate as hurdles in combination with pH and moisture content on pickling, powdering, packaging, quality attributes and stability of these two buffalo meat products.

The specific objectives of the present investigation were as listed below:

* Development of convenience buffalo meat products namely pickles (Fig. 1) and powder (Fig. 2) using a combination of selected hurdles viz. pH, moisture content, chemical preservatives. spices/condiments and packaging materials.
* Evaluation of microbiological, physico-chemical, textural and organoleptic characteristics of developed buffalo meat products.
* Evaluation of shelf stability of the developed products and changes in their characteristics, especially microbial characteristics, during storage at ambient temperature in different packaging materials.

Keeping in view the above objectives, the effects of pickling medium and preservatives, materials of packaging, methods of packaging (in case of meat powder only) and period of storage were examined on microbiological, physico-chemical, textural and organoleptic characteristics of developed products and their shelf stability.

Fig 4.1 Oil based meat pickles packed in HDPE and glass jars.

Fig 2 Dehydrated buffalo meat powder in combination film and autoclaveable polythene packed by atmosphere, vacuum and MAP packaging methods.

Pre-rigor meat consisting of round portions comprising mostly of semi-membranous, semi-tendenous bicep femoris and quadriceps muscles of male carcass of buffalo, slaughtered by 'Halal' method was used in the study. The animal was slaughtered 2h before the sample collection. The meat samples were packed in HDPE bags and brought to laboratory within 10 minutes after separation from carcass and immediately stored in a deep freezer at 4 ± 1°C. The bicep was trimmed off and meat samples were cut into chunks of 2.5 to 3.5cm size before conducting further studies .

Although, the weight, sex, source, method of slaughtering and collection of different meat samples, procured on different days during experiments were tried to be kept same. Anticipating qualitative changes in meat, its proximate composition were experimentally determined. The average values for pH, moisture, protein, fat and ash contents and thiobarbituric acid (TBA) of fresh meat were determined at 5.89, 75%, 19.32%, 10.29%, 1.0% and 0.3 mg/kg, respectively.

For development of meat pickles, the similar recipe as recommended for preparation of vegetable pickles was used. The untreated control as well as the meat pickles treated with 2% each of cinnamon, clove, turmeric, garlic, 0.025 % of potassium sorbate and 0.02 % sodium nitrite were developed in soybean oil medium. However, the mustard (2%) and acetic acid (6 %) treated meat pickles were developed in their own mediums.

The various microbiological characteristics viz. total plate count (TPC), coliform count, lipolytic count, acidophilic count and yeast and mold counts, physico-chemical characteristics (pH, moisture content, protein, ash content and thiobarbituric acid (TBA) values), textural characteristics (hardness, cohesiveness and gumminess) and organoleptic characteristics (colour, odour, texture, taste and palatability) of both the fresh and preserved meat pickles were determined following standard laboratory methods. Organoleptic evaluations of both products were carried out with the help of

a six member panel of semi-trained panelists drawn from the laboratory/department using a nine point hedonic scale.

For storage and shelf life stability studies, the samples of all pickles developed in various mediums and treated with spices, acetic acid, potassium sorbate and sodium nitrite, were preserved in HDPE and glass jars by atmospheric packaging method. The packed samples were stored for 120 days at ambient conditions during March to September when the temperature ranged between 30 to 35 °C.

The data of all experimental studies were statistically analysed for variations among the mean values of each individual parameters. The two ways ANOVA for each quality parameter was carried out to find out any significant difference between the mean values.

For development of another hurdle processed meat product, "the meat powder", raw buffalo meat was dehydrated and transformed into powder form. Dehydration of meat was carried out in a tray drier at 180°C to 60°C for 3 days. The dehydrated meat pieces were powdered in a grinder. For stability studies, the developed powder samples were packed in two different packaging materials namely combination film and autoclavable polythene. In this study, the hurdle parameters were incorporated also in the form of treatments. To increase the shelf life, the meat after dehydration was treated with clove, turmeric and potassium sorbate. Three other treatments used were related to packaging. All the samples were stored at ambient temperature during August to February when the atmospheric temperature ranged between 35°C to 12°C. The various microbiological, physico-chemical and sensory characteristics of preserved meat powder samples were evaluated by using standard techniques. To test the functional suitability of preserved meat powder, the powder was reconstituted and used for developing Shami-kababs. The sensory characteristics of developed kababs from preserved meat powder samples were also evaluated. All

the data were statistically analyzed. The results characteristic are summarized as under:

PRODUCT ONE: HURDLE PROCESSED MEAT PICKLES

* Treatment of meat with certain natural products (spices and condiments) and synthetic chemicals as hurdles parameters during pickling resulted in differential reduction in pH in a time-dependent manner during 120 days of storage at ambient temperature. Spices used as natural preservatives, particularly the cinnamon, clove and garlic significantly reduced the pH values of pickles with in 20 days of storage vis-à-vis untreated control and synthetic preservatives, except acetic acid. Treatments with the various preservatives resisted the change in pH of meat pickle in the order as potassium sorbate > sodium nitrite > mustard > turmeric > garlic > clove > cinnamon > acetic acid. The low pH in acetic acid, cinnamon, clove and garlic treated meat pickles prevented the spoilage and extended the shelf life of the product by retarding microbial growth.
* Pickling treatments with natural products as preservatives have added to the protein content of meat as compared to control. The protein values of meat pickles increased in the order as clove > turmeric > cinnamon > garlic > mustard. The protein content of treated pickle remained unchanged upon storage, however, the decrease in protein content in control meat pickle was noticed during 120 days of storage. The results suggested the beneficial effects of spices and condiments on stability of meat pickles.
* Pickling treatments with natural and synthetic preservatives maintained the TBA number of meat during storage, however, it increased substantially as a function of time in case of control during storage at ambient temperature. Slight increase in TBA values was noticed in garlic, mustard, potassium sorbate, and sodium nitrite treated pickles. Nevertheless, the TBA values invariably lie with in the threshold limit of

1mg/kg at which lipid rancidity occurs. Comparative analysis revealed that the meat pickles packed in glass jars exhibit lower TBA values than the HDPE jars.

- The ash contents in both control and treated meat pickles with spices / condiments were found to increase significantly as compared to raw meat and did not changed with storage. Insignificant effects of storage period and packaging material on ash content of pickles were observed.
- The effective concentrations of spices/condiment and synthetic chemicals used in the study were determined based on their MIC value against the pure cultures of common bacteria associated with meat spoilage. The MIC of the extracts of spices/condiments against *Staphylococcus aureus, Salmonella enteritidis, E.coli* and *Listeria monocytogenes* were determined to be 2%. However, the MIC of synthetic preservatives was determined to be 0.02%, 0.025% and 6% for sodium nitrite, potassium sorbate and acetic acid, respectively. Both the natural and synthetic products at these sub-lethal concentrations were applied as treatments to meat pickles.
- Pickling medium, preservatives, storage condition and time period have significantly influenced the microbial quality of meat pickles. Nevertheless, in all cases the pickles were in edible condition (hedonic rating 5.67 to 8.67) even after 120 days storage at ambient temperatures. Significant reduction in microbial population (TPC) was observed as a function of storage time in all treatments irrespective of packaging material. On the contrary, the TPC increased in untreated control and potassium sorbate treated meat pickles in both packaging materials.
- The efficacy of natural products (spices/condiments) for microbial control was observed to be in the order as clove > garlic > turmeric > cinnamon > mustard. Amongst the synthetic preservatives, the efficacy was in the order as sodium nitrite > acetic acid > potassium sorbate. Packaging of pickles in glass jar was found to be safer than HDPE jar for long term storage under ambient conditions.

- Treatment of meat with both the natural and synthetic preservatives significantly reduced the coliform counts in pickles. However, pickles treated with mustard and potassium sorbate exhibited recurrence of *E.coli* growth after 20 days of storage at ambient temperature, which increased further with increase in storage period. Glass jar was found to be a better packaging material for long term storage of meat pickles. Cinnamon, clove, turmeric and garlic treatments exhibited effective anti-microbial activity, and suppressed the growth of coliforms during long term storage.
- Treatments with the natural products as preservatives resulted in significant decrease in *Staphylococcus* count in meat pickles after 20^{th} days of storage. Amongst synthetic preservatives, sodium nitrite and acetic acid suppressed the growth of *Staphylococcus* group of bacteria. However, potassium sorbate treatment increased the count during storage. Glass jar was suggested to be better a packaging material than HDPE jar.
- Treatment of meat pickles with clove, turmeric, cinnamon, effectively also checked the growth of proteolytic microorganisms. Moreover, all five natural products at 2% level viz. cinnamon, clove, turmeric, garlic and mustard inhibited the growth of lipolytic microorganisms. The synthetic chemicals viz. sodium nitrite, potassium sorbate and acetic acid also caused 100% inhibition of the growth of lipolytic bacteria up to 20 to 40 days. The lipolytic bacteria, however, reappeared upon prolonged storage at room temperature.
- Treatments of meat pickles with cinnamon, clove, turmeric, acetic acid and potassium sorbate were effective in controlling the growth of yeast and mold counts during 120 days storage. However, garlic in meat pickles was not effective in checking the growth of yeast and molds count.
- The pickles treated with all natural and synthetic preservatives resulted in the products, which were placed in 'liked very much' category based on sensory evaluation, as far as the colour is concerned. The sensory score, however, decreased

during storage. Glass jar proved better than HDPE jar in retaining the colour of pickles during storage.

- The odour scores of pickles increased significantly with storage period. All pickles except acetic acid treated pickles were rated as 'liked very much' on odour scores. Mustard and clove treated pickles were most liked while turmeric and acetic acid treated pickles were least liked. From odour score point of view, also glass jar was better than HDPE jar for long term storage of pickles.
- Invariably all the treatments significantly influenced the texture of pickles during storage at ambient temperature. The storage significantly improved the texture of all pickles. On the 120^{th} day (at the end of storage) cinnamon, turmeric and mustard treated pickles had the highest score for texture which was liked very much. Acetic acid pickles, however, were slightly disliked. No significant effect of packaging material on textural qualities of meat pickles was observed. Treatment with clove and cinnamon and acetic acids significantly reduced the hardness of the pickles.

PRODUCT TWO: HURDLE PROCESSED MEAT POWDER

- The meat powder had average particle size of 0.26mm, moisture content of 4.0 to 4.2%, pH of 5.39 to 6.03, protein content of 67.1%, fat content of 11.77% and TBA number of 0.29 ± 0.07mg/kg and exhibited a shelf life of 120 days.
- The moisture content, pH, fat content and ash content of meat powder did not significantly changed during storage upto 120 days at ambient temperature. The treatment of meat before drying and powdering had insignificant effect on pH and protein content of meat powder.
- The two packaging materials used for meat powder namely combination film and autoclavable polyethylene also had insignificant effect on pH and protein content of meat powder.

- Meat dehydration and powdering significantly increased the fat content of meat product. Treatment of meat powder with different preservatives after dehydration had insignificant effects on fat content of powder, as was the case with two packaging materials used for storage of meat powder.
- Treatments of meat after dehydration and powdering significantly increased the TBA number of meat powder during ambient storage, to extent that the product crossed the threshold level of rancidity, probably due to degradation of fatty acid.
- Meat powder treatments with clove and turmeric after dehydration and subsequent powdering completely checked the TPC of meat powder. The powder remained microbe free virtually with no detectable *Staphylococcus*, proteolytic or lipolytic bacteria throughout 120 days storage period. The bacterial count, however, increased in untreated control meat powder. Autoclavable polyethylene as packaging material in comparison to combination film was observed as inappropriate packaging material. Vacuum packaging was assessed to be better packaging alternative for maintaining low microbial count vis-à-vis modified atmosphere packaging of meat powder.
- Yeast and mold counts in untreated control meat powder have significantly increased during storage for 120 days. However, treatment with clove effectively controlled the growth of these microorganisms. Turmeric treated meat powder exhibited some yeast and mold growth after 60 days storage in the powder packed in autoclavable polythene. Potassium sorbate completely prevented the growth of yeast and mold in both packaging materials. As compared to CO_2 and N_2 flushing methods, the bacterial and fungal growth control was very good in vacuum packed meat powder.
- The developed meat powder was rated as 'liked very much' with respect to colour and odour. Treatments of meat significantly improved organoleptic characteristics. Vacuum packaging was the best method as compared to other methods of packaging.

- The Kababs developed from meat powder preserved for 120 days were rated as 'most liked' because of good organoleptic scores.
- To the best of author's understanding, the present investigation is the first effort in global scenario for preparation, preservation and utilization of buffalo meat in powdered form. Such a product could be stored safely at ambient temperature for 120 days using spices/condiments as preservatives, maintaining the low TBA value and microbial counts. Also, this unique method of meat preservation exhibits enormous potential of utilization of meat for a long duration and faster reconstitution to produce variety of meat products viz. kababs, patties and balls of varying dimension.

CONCLUSIONS

Based on above studies, following conclusions are drawn:

Pickling of fresh buffalo meat and drying–cum–powdering of buffalo meat, using selected hurdle parameters viz pH, preservatives, dehydration, and packaging, etc. proved to be effective methods for controlling the meat microflora and preserving the meat for subsequent long term consumption.

The pickling medium (soybean/mustard oil), spices, organic acids and salts, packaging material and methods, storage temperatures, etc. create a distinctive micro-environment in and around the meat products, which inhibits the proliferation of the deleterious microflora responsible for meat spoilage. Pickling treatments and subsequent fermentation contribute to improvement in flavour and textural properties. The primary fermentation product lowers the meat pH and contributes to the stability of these pickles against food borne pathogens and other less desirable spoilage microorganisms.

Among the spices used in present study, the role of garlic, turmeric and clove in management of diabetes mellitus and lipid metabolism, and as anti–inflammatory agent, respectively are well documented. Development of meat pickle with these

spices adds medicinal value to product as a bonus. These spices are also known to stimulate pancreatic digestive enzymes such as lipase, amylase and protease, which may play a crucial role in digestion and reduction in food transit time in gastrointestinal tract.

Although, the spices/condiments used in present study were initially selected as hurdle parameters owing to their antimicrobial activity, to extend the shelf life of products (pickles and powder), however, the correlation of data with inherent characteristics of spices outspread their efficacy. The spices/condiments used in preparation/preservation of meat pickles and powder could exhibit multi-pronged benefits as (i) antimicrobial preservative, (ii) flavouring agent (iii) natural therapeutic agent with the host of beneficial physiological effects. Keeping in view many promising health beneficial effects, such food adjuncts could be regarded as 'neutraceuticals', which not only makes the food spicy but healthier too.

Thus, both the hurdle processed meat products namely pickles and powder have the advantage of a longer shelf life (120 days), desirable organoleptic attributes, safe ingredients and low cost of processing. The longer shelf life of both the products at ambient temperature and good nutritive/medicinal values may add great convenience to many meat consumers. Both the products could also serve as protein supplement for the defense establishments, hotels/ restaurants/ fast food shops, and travelers etc. due to the logical advantages of 'easy to pack', 'easy to cook' and 'ready to eat' properties.

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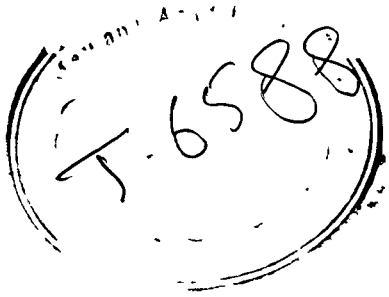
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DEPARTMENT OF AGRICULTURAL MICROBIOLOGY
FACULTY OF AGRICULTURAL SCIENCES
ALIGARH MUSLIM UNIVERSITY, ALIGARH- 202002, INDIA
Tel. (0571) 2703516, 2700920 EXT 260 (o), 2502283 (R)
Fax 91 –571-2703516 Telex 564-230 AMU IN

Certificate

This to certify that the research work embodied in this thesis entitled ***"Development preservation and microbiological quality evaluation of hurdle processed buffalo meat products under ambient conditions"*** *is an original work, unless otherwise stated, carried out by Ms. Mahjabeen Siddiqui under our supervision, and is suitable for submission for the award of Ph.D degree in Agricultural Microbiology of the Aligarh Muslim University, Aligarh.*

19/10/05

Prof. Javed Musarrat
Chairman
(Supervisor)
Deptt. of Agricultural Microbiology
F/O Agricultural Sciences
A.M.U Aligarh

Prof. P.K. Srivastava
Chairman
(Co-supervisor)
Deptt. of Post Harvest Engg. & Tech
F/O Agricultural Sciences
A.M.U Aligarh

Declaration

I, hereby, declare that the work embodied in this thesis entitled "Development preservation and microbiological quality evaluation of hurdle processed buffalo meat products under ambient conditions" is carried out by me

Ms. Mahjabeen Siddiqui
Ph.D Student

CONTENTS

Dedicated To My Loving Parents

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List of Abbreviations

ADP	Adenosine diphosphate
AMU	Aligarh Muslim University
ANOVA	Analysis of variance
a_w	Water activity
ATP	Adenosine triphosphate
^oC	Celsius
CD	Critical difference
CO_2	Carbon dioxide
DFD	Dark, firm and dry
df	Degree of freedom
E_h	Oxidation-reduction potential
ERV	Extract release volume
FAO	Food and agricultural organization
F	Variance ratio
FDA	Food and drug administration
HACCP	Hazard analysis of control critical point
HDPE	High density polyethylene
ICMR	Indian council of medical and research
kg	Kilogram
MAP	Modified atmosphere packaging
mm	Millimeter
mss	Mean sum of square

N_2	Nitrogen dioxide
NaCl	Sodium chloride
ND	Not detected
NPN	Non–protein nitrogen
PCR	Polymerase chain reaction
P	Level of confidence
PE	polyethylene
PET	Polyethylene terepthalate
PPF	Positive peak force
RH	Relative humidity
SD	Standard deviation
ss	Sum of square
TAHD	Texture analyzer heavy duty
TBA	Thiobarbituric acid
TCA	Trichloro acetic acid
TPC	Total plate count
TVC	Total variable count
TV	Tyrosine value
WHC	Water holding capacity
Y&M	Yeast and mold

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Introduction

Meat and its products scientifically refer to the muscles of warm-blooded terrestrial four-legged animals viz. cattles, goat, sheeps and pigs. It also includes the glands and organs of these animals in addition to many of the by-products of animal slaughter such as gut, fat and gelatin. Based on its composition, meat is regarded as concentrated source of many nutrients. It is rich in proteins containing all the essential amino acids, minerals and vitamins and, therefore, considered as an important food, which is being consumed since times immemorial. Meat is considered as a very valuable source of high biological value protein, which is tasty, palatable and easily digestible. It stimulates secretion of gastric juices, though excess meat consumption, however, is discredited with ailments such as gout, carcinoma and hypertension, etc.

The quality of meat is the most important criteria from the consumers point of view. The quality attributes of meat include its physico-chemical characteristics, microbial profiles and organoleptic characteristics such as colour, taste, texture and juiciness, etc. Scientifically, the meat quality includes atleast seven characteristics such as wholesomeness, nutrition, processing yields, convenience, consistency, appearance and palatability. The palatability has five components viz. tenderness, texture, juiciness, flavour and taste.

Meat proteins are good source of carbon and nitrogen that are essential for microbial growth. Microorganisms degrade the muscle proteins by proteolysis and utilize the essential nutrients for their growth during spoilage process, which depends upon the composition of muscle and type of microflora on meat (Gill and Newton, 1982).

Raw meat is highly perishable in nature and needs to be preserved and processed. Out of several factors responsible for deterioration of meat viz. microorganisms, enzyme activities, moisture content, temperature, and light, the microbial factors are of special significance as some of these are resistant to most

processing conditions. Some of the microorganisms are very harmful and result in undesired fermentation. They multiply best between 16 and 38°C, while some grow at 0°C and others at a temperature as high as 100°C. They can hydrolyse lipids and produce rancidity while others digest proteins and produce putrid, rotten flavour and ammonia like odour. Bacterial food borne spoilage, predominantly occur due to food infection or intoxication. The former type of spoilage is on account of organisms present in meat (food) at the time of consumption, which grow in the host and cause diseases. The later type of spoilage is due to toxins produced by bacteria prior to consumption, which causes fatal effects upon ingestion. For instance, the toxin of *C.botulinum* is produced only under anaerobic conditions such as meat stored in an airtight packages. Frozen meat does contain such microbes but their growth is restricted. Food preservation, in this reference demands the killing of various microbes or its inhibition besides lowering the biochemical changes with least changes in organoleptic and nutritional characteristics.

Because of varied sources, the kinds of microorganisms likely to contaminate meat and meat products are many. Molds of many genera may also reach the surface of meat and meat products and exhibit growth. Especially important are the species of the genera *Cladosporium*, *Sporotrichum*, *Geotrichum*, *Thamnidium*, *Mucor*, *Penicillium*, *Alternaria*, and *Monilia*, etc. There is also possibility of contamination of the meat and its products with human pathogens, especially those of intestinal type in the retail market. Variance tools such as knives, saw, cleavers, grinders, chopping blocks, scales, containers, etc. as well as the market operators may serve as the source of microbial contamination.

From the spoilage point of view, the processing and preservation of meat has been the subject of interest since beginning of the civilizations. The original concept for processing of meat was only its preservation by inhibiting or deterring microbial

decomposition in order to prevent its spoilage. However, in present days, it is considered as a method, which provides convenience as well as variety to the meat portion of the diet, in addition of preservation. The term processing of meat in present days includes all processes, which are utilized in altering fresh meat. In broadest sense it excludes operations like simple grinding, cutting and mixing but includes operations like curing, smoking, freezing, canning, cooking, dehydration, use of certain chemicals and enzymes, production of intermediate foods, etc. Some of these operations, however, either incur some additional cost or alter some of the desired qualities of it or both. Increased demand for convenience food has resulted in expansion of processed meat industry. This has been accelerated by the relatively large number of women getting employed out side the house and the consequent lack of time available for preparation of meals. Since meat has always required the longest period for preparation, it has benefited most from development of convenience items. This is best illustrated by the growth of 'heat and eat' meals, such as frozen dinner, where meat as the entire is the major contributor to the meal. Luncheon meats, such as frank furters, bologna, meat loaves, etc. have also been major beneficiaries of the increased use of convenience foods.

It seems that the trend towards complete processing for consumers convenience and development of new meat products will continue resulting in more prepared and pre-cooked items. This, in turn, will determine the future of the processed meat industry. The recent trend is in reducing the fat and salt (sodium) contents of muscle based foods. Also, the demand for so called 'healthy foods' has led to the development of 'low fat' and 'low salt' meat products. This requires alteration of traditional preservation and processing methods and use of a number of non-traditional procedures and additives in order to achieve these aims besides maintaining the acceptability of meat products.

Amongst various traditional methods of meat processing, grinding appears to be attractive from the economic point of view, which may led to a variety of comminuted, convenience and value added meat products. However, grinding of meat leads to (i) rapid formation of metmyoglobin, (ii) the undesirable brown colour and (iii) oxidative rancidity which seriously affects the consumer acceptance. Preservation of meat by application of heat is one of the most acceptable and effective alternative methods. The purpose of heat treatment is to kill those microorganisms, which inactivate the enzymes in meat that may become active during subsequent storage of processed and packed meat food. Cooking of meat by application of heat can make meat more or less tender than the original raw cut, though there may be some toughening influences of heating. Over heating, however, can cause muscle fiber to contract and meat to shrink and become tougher. Evaporation of moisture also occurs as a result of which dried out tissues become tougher. Lower cooking temperature for a longer period of time is, therefore, considered better than a higher temperature for a short period of time for any degree of doneness. This results in decreased drip losses, less shrinkage, increased juiciness and more uniform colour through the cut. Thus, the control of temperature is very important as on one hand, too high temperature not only causes the fat of meat and cooking medium to smoke but also to be underdone in the middle while the outside may have a pleasing colour. On the other hand, if the temperature is too low, it will lengthen the cooking time and result in a greasier product. When fresh meat is cooked, it's protein pigment are denatured, which in turn causes rapid release of the haem, which is very sensitive to oxidation. This is why red meat generally turns brown due to oxidation of pigments. The enzymes present in meat are inactivated due to heat and such inactivation of proteins like myosin and actin brings textural changes. Protein and free amino acids of meat on heating produce some volatile breakdown products. These include sulphur containing compounds,

aldehydes, ketones, alcohols, amines, and others. Lipid components also break down into various volatile components. These volatile components in both the fat and lean portion of the meat, contribute to the flavour and odour of the cooked meat. There is loss of some B- complex vitamins during cooking, though most of the cooked meat retain more than 50% B- complex vitamins present in cooked meat. However, the nutritional value of cooked meat is high though such cooked products are also dehydrated or freeze dehydrated to limited extent.

As meat in India is handled at ambient temperature for a relatively longer time due to lack of refrigeration facility, it may lead to microbial contamination and spoilage, which results in huge economic losses and poses public health hazard. Since the meat is an excellent medium for growth of microorganisms, poor hygienic production and processing procedures, inadequate storage conditions and lack of processing facilities may provide ample opportunities for microbial decomposition. An important challenge, therefore, faced by meat scientists in India is to develop technology for safe, wholesome, palatable, and convenient products, which offer real value for money. This is why research recourses are being focused on the development of profit enhancing technology in processes and product development.

In above reference, the multi-targeted approach of hurdle technology is considered as an effective and economically viable process of meat preservation, as it insures an optimal combination of various preservation factors known as hurdles (Berwal, 1994).

There are several preservation factors viz. temperature, water activity, acidity, preservatives, packaging materials, etc. which act as hurdles to inhibit the growth and activity of microorganisms. These hurdles disturb the homeostasis, which is the constant tendency of microorganisms to maintain a stable, uniform and balanced

internal environment. Disturbance of the homeostasis prevents the multiplication of microorganisms and causes them to remain non-viable.

The hurdle technology avoids harshness or drawbacks of a single preservation technique (Leistner, 1978 ; 1985 ; 1992 ; 1995). Each hurdle, which is not sufficiently intensive gives treatment to meat products to cause destruction of microorganisms or spores due to sub-lethal damage, which may be augmented by other hurdles in an additive manner and through synergistic manner (Brimelow, 1985; Leistner, 1994 ; Grigspaardt, 1994). Application of hurdle technology also helps in improving the product quality. The products may be shelf stable and possess superior quality with almost fresh like characters. Evidences also show that moist foods developed on the basis of hurdle technology are stable without refrigeration. This concept of storage of high moisture food without refrigeration seems to have great potential particularly in most tropical countries. However, as from the viewpoint of controlling microbial spoilage and undesired fermentation process, which affect its quality, quantification of the influences of each hurdle and its levels on the microbial as well as nutrient stability of food is an important research area in food product design.

The concept of hurdle technology has been applied by various researchers on various food products (Hechelmann et al., 1991; Leistner, 1985 ; Manish and Berwal, 1996; Karthikeyan,1997; Wang and Leistner, 1993 ; Modi et al., 1999 ; Himanish and Sumithara, 1998 ; Das and Radhakrishna, 2001). The hurdle technology based food preservation has proven to be a useful approach for development of foods industry, combining the advantages of predicted microbiology and Hazard Analysis of Control Critical Point (HACCP). In industrialized countries, the applications of hurdle technology has been made for development of minimally processed convenience foods, chilled food with 'in-visible' technology, health-full foods with less salt and /or fat, less packaged foods. In developing countries, though the technology has not been

utilized, its application may be useful in development of novel foods as well as in modification of traditional intermediate moisture foods to make them shelf stable. An added advantage of hurdle technology in this reference is that it is applicable both in large and small food industries.

There are, however, several instances of use of individual hurdles in preservation and processing of meat and meat products. For example, the water activity (a_w) can be reduced by dehydration, cooking or addition of humectants. Dehydration and cooking, both help in reducing microbial load of the product. Several acidulate organic acids like citric acids are used to reduce pH of the meat. Moreover, addition of citric acid enhances the inhibitory property of potassium sorbate against *C.botulinum* (Webster and Cook, 1984). As an antimold agent, potassium sorbate added to beef reduces the contamination remarkably (Zaemora and Zartizky, 1987) and prolongs the shelf life. Similarly, some spices and condiments, used in meat preservation are known for their antimicrobial and antioxidant properties (Chipault et al., 1955; Kikuzaki and Nakatani, 1993; Rajlakashmi and Narsimhan, 1996). These studies suggest the posibility of producing hurdle processed meat products in cost effective manners in India, both for domestic consumption as well as for export.

As far as meat production is concerned, India is bestowed with abundant livestock resources (Table 1.1), which account for about 11% of the world livestock population (Kumar et al., 2002). From these resources, the country produces about 4.7 million tonnes of meat (Anon, 2001) as shown in Table 1.2. The total export of meat and meat products (including poultry products) from India is presented in Table 1.3.

From Table 1.2, it may be noted that buffalo contributes to about 30.27% of total meat production in country. The buffalo meat is preferred in various parts of the world because of its better eating qualities. Unfortunately, in India the buffalo meat is mostly produced from unproductive and old animals (Sahoo et al, 2000). The meat is

Table 1.1 Estimated livestock population in India

Species	Population (in Million)	Rank in world
Buffalo	80	1
Sheep	51	5
Goat	123	1
Pig	12	--
Cattle	206	1
Poultry	435	--

(--) not available
Adapted from: Kumar et al. (2002)

Table 1.2 Production of meat by source (in tonnes)

Meat sources	Years		
	1996	**1999**	**2000**
Beef/ veal / kine	1,401	1,421	1,442
Buffalo	1,380	1,410	1,421
Goat/sheep	688	694	696
Pork	542	560	560
Poultry meat	540	559	575
Total meat	4,551	4,644	4,694

Adapted from: Anon (2001)

Table 1.3 Export of meat and poultry products

Year	Export worth (Rs. in Crore)
1995-1996	650.01
1996-1997	788.23
1997-1998	850.22
1998-1999	859.00
1999-2000	845.00
2000-2001	860.04

Adapted from: Ministry of Food Processing (2001), Annual Report Industries, Govt. of India

coarse, tough in texture and dark in colour. However, such meat could be profitably utilized by comminuting and using in a variety of convenience and value added meat products (Anjaneyulu et al., 1989 ; Govindarajan, 1973 ; Kondiaha et al., 1988 ; Sahoo, 1989 ; Sahoo et al., 1998). Nevertheless, grinding of such meat leads to rapid discolouration and warmed off flavour due to pigment and lipid oxidation, which seriously affects the consumer acceptance. To minimize the oxidation problem, many chemicals preservatives have been tried (Sahoo and Anjaneyulu, 1997).

The application of hurdle technology in preservations and processing of such tough and coarse meat has been explored. However, extensive and systematic investigations are needed to address the queries such as: *could the hurdle technology be utilized to develop new buffalo meat products ? If so, what are the characteristics of such products ? Are they shelf stable ? What are their sensory characteristics ?, etc.* The available literature on these aspects is scanty. Practically no information is available with respect to use of hurdle technology in case of processing and preservation of buffalo meat. The individual technique or hurdle have been studied but not as a combined hurdle technology, which is the major research gap.

Keeping the above issues in mind, the present investigation was carried out to develop two non-traditional buffalo meat products viz. pickle and dehydrated meat powder and to determine their characteristics and shelf life. The specific objectives of the investigation were:

(I) Development of convenience buffalo meat products namely pickles and powder using a combination of selected hurdles viz. pH, moisture content, preservatives, acids, spices/condiments and packaging materials.

(II) Evaluations of microbiological, physico-chemical, textural and organoleptic characteristics of developed buffalo meat products.

(III) Evaluations of shelf stability of the developed products and changes in their characteristics, especially microbial characteristics, during storage at ambient temperatures when packed in different packaging materials (using atmospheric, vacuum and modified packaging in case of meat powder).

Review of Literature

This chapter presents a detailed review of literature related to topics of importance to the present research investigation. It includes information on definition and types of meat, its structure and composition, microbiology and nutritional characteristics, spoilage and causes of spoilage of meat and preservation methods, etc. Special emphasis has been given on use of individual hurdle parameters for preservation of two non-traditional products from buffalo meat namely pickle and dehydrated meat powder, which were developed in the present investigation as preserved meat products.

2.1 Meat

Scientifically, the term meat and meat products refers to the muscle of warm-blooded terrestrial-four legged animals viz. cattle, sheep and pig, etc. It also includes the glands and organs of these animal in addition to many of the by-products obtained from slaughtered animals such as animal gut, fat, gelatin, etc.

In meat processing industry different terms are used to designate the meat from different types of animals. For example: veal is the meat from cattle, slaughtered three to four weeks after birth. The carcass of 14-52 weeks old cattle is called calf while beef is normally the term used to refer the meat of cattle over one year old. It may be further classified as stear, heifer, stag, etc. The meat of sheep and goat are normally called mutton while pork and buffalo meat refer to meat of swine and buffalo, respectively. Organ meats include liver, kidney, heart, sweat bread (thymus and pancreas), brain, lung, head, tail and tripe (first and second stomach of the ruminants).

2.2. Structure of meat

Skeletal muscle is composed of long cylindrical, multinucleated cells (fibers) which are arranged in a parallel fashion to form bundles, held together by connective tissues. The connective tissues merge at the terminal of the muscle to form a tendon,

which in turn connects the muscle to bone. The surface of the muscle, called the 'sarcolemma' is composed of three layers: an outer network of collagen fibrils, a middle amorphous layer and inner plasma membrane. Invaginations of the plasma membrane form the transverse system, which has the function of extending the plasma membrane into the interior of muscle.

The semi-fluid material within the muscle cell, called the "sarcoplasm", contains soluble components such as myoglobin, some enzymes and some metabolic intermediates. Glycogen particles and lipid droplets also occur in some muscle cells, depending upon the state of muscle. Sarcoplasm, in addition, contains other constituents including the contractile apparatus.

Each muscle fiber is composed of many parallel smaller fibrils (myofibrils) constituting 60% of muscle. Approximately, 2000 myofibrils, 1.0 to 3.0 μm in diameter, are found in an average sized fiber. The characteristic striated appearance of skeletal muscle is due to a specific repetitive arrangement of protein in the myofibrils. When viewed under a microscope they show alternate dark and light bands. The dark bands are termed as anisotropic (A) bands, and the light bands as isotropic(I) bands. In the centre of each of the I band is a dark line, called the Z line. The centre of the A band, called the H zone, is lighter than the rest of the A band. Usually, in the centre of the H zone there exists a darker. 'M'. line. The contractile unit is called 'sarcomere' and is the material located between and including two Z lines. The structure of skeletal muscle and muscle fibre are shown in Figs 2.1 and 2.2, respectively.

The A band consists of thick and thin filaments and the I band of the thin filaments. The thin filaments are attached to the Z-line and they extend outwards in both directions of the line. In parts of the A band the thin filaments overlap the thick filament. The lighter zone in the A band (H-zone) is the area where the thick and thin bands do not overlap, i.e. the H band consists of only thick filaments.

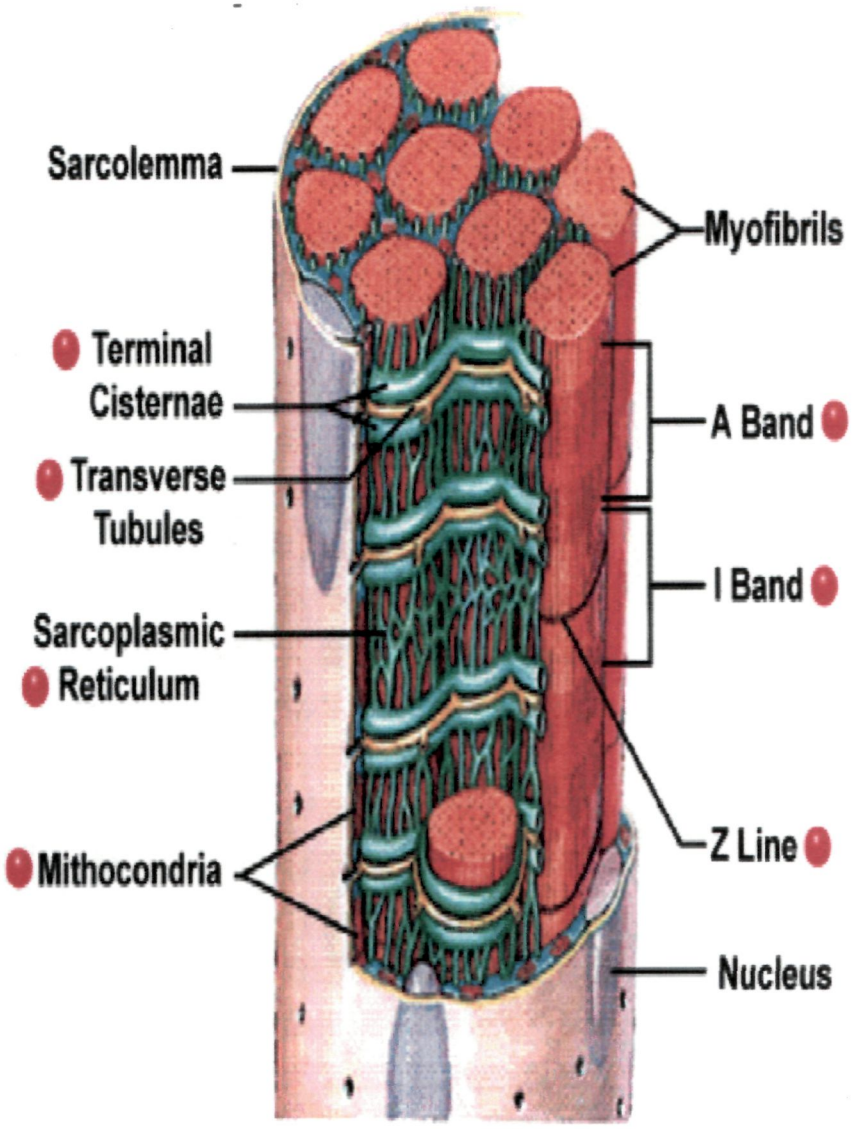
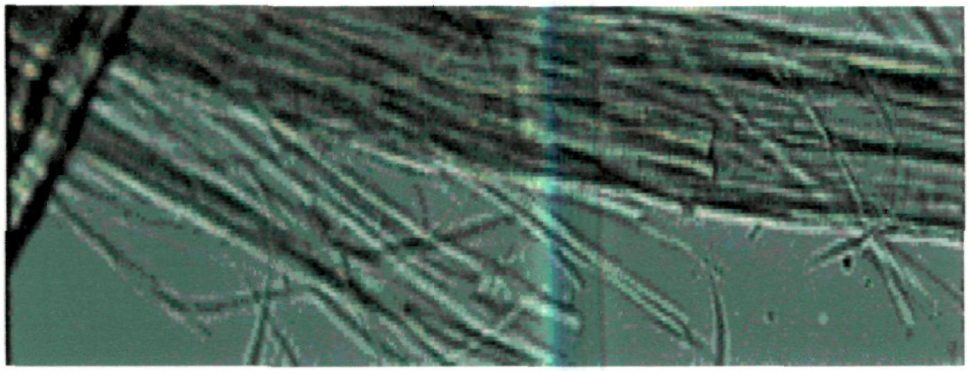
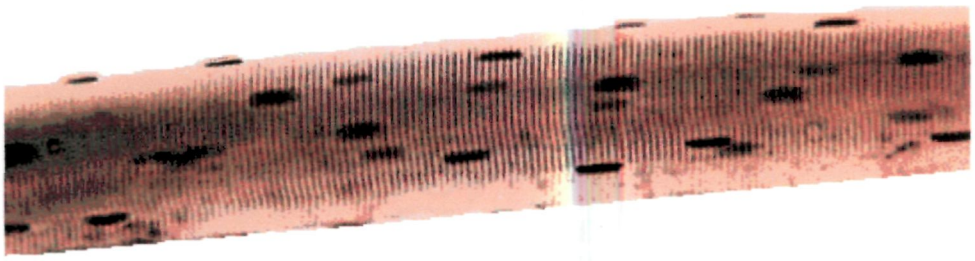


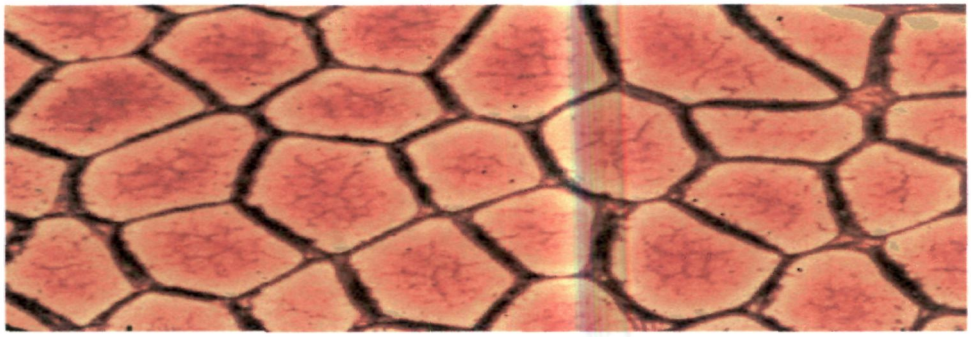
Fig 2.1 Structure of skeletal muscle.



(a)



(b)



(c)

Fig 2.2 Structure of muscle fibre
(a) Muscle fibre structure observed with an ordinary light microscope
(b) A short part of one muscle fibre, containing many nuclei (the dark blobs)
(c) Endomysium in a transverse section of meat (the endomysium is black and myofibrils are yellow)

The thin and thick filaments slide paste each other, and facilitates the contraction of muscle. During contraction, the length of the A band remains constant, while both the I band and H zone shortened.

2.3. Composition of meat

The lean meat contains 20 to 22 % proteins. The total nitrogen content of meat comprises of 95% protein and 5% smaller peptides and amino acids. The fat content of meat varies from 5 to 40% with the type, breed, feed and age of the animal. When the animal is well-fed, fat deposits subcutaneously as a protective layer around the organs. It also accumulates around, and between the muscles. The fat penetrates between the muscle fiber bundles and is referred as "marbling". Marbling is desirable with some meats (like beef) because the amount of fat and the water holding capacity of the meat, greatly influence juiciness. Meat fat is rich in saturated fatty acids, and it is likely that it produces certain forms of atherosclerosis. The cholesterol content in meat is about 0.75%. The lean portion of meat contains greater proportion of phospholipids (0.5 to 1.0 %), and these are located in the membranes of the cell. The fatty acids in the lean portion of meat have a higher proportion of unsaturated fatty acids than tissue fat. Carbohydrates are found only in very small quantities in meat. The two carbohydrates found in meat are glycogen and glucose.

Meat is an excellent source of some vitamins of the B-complex, iron, phosphorus and minerals. Meat also contains sodium and potassium. The approximate composition of meat is given in Table 2.1. Meat also contains the protein hydrolyzing enzymes like cathepsins, which are responsible for increased tenderness of meat during ageing. The three types of meat proteins namely contractile, soluble and insoluble proteins, are described below:

(i) Contractile proteins

These proteins are soluble in salt solutions of high concentration and are of

Table 2.1 Composition of meat of different animals per 100 g of edible portion

Name of flesh foods	**Moisture (g)**	**Protein (g)**	**Fat (g)**	**Minerals (g)**	**Energy (Kcal)**
Beef *Bos taurus*	74.3	22.6	2.6	1.0	114
Mutton muscle	71.5	18.5	13.3	1.3	194
Pork muscle *Sus cristatus Wagner*	77.4	18.7	4.4	1.0	114

Adapted from : Nutritive Value of Indian Foods,NIN,Hyderabad,ICMR, India,(1984)

following sub-types.

(a) Myosin

This is the major protein of the thick filaments, and contains two identical polypeptide chains bound around each other and constituting the 'tail'. The myosin molecules develop polarity with globular regions on either side and the straight portion in the centre when they interact, joining in head to tail fashion. The polarities of the myosin molecules are reversed on either side of the centre but all molecules on the same side have the same polarity. It is this polarity, which allows contraction to occur.

(b) Actin

This is the major protein of the thin filaments and constitutes 15 to 30% of the myofibrils.

(ii) Actomyosin

Actin and myosin interact to form actomyosin. This interaction is responsible for muscle contraction. The interaction of actin and myosin in the absence of enzyme activity influences the quality of meat.

(iii) Other contractile proteins

Tropomyosin, troponin, α actinin and β actinin are other proteins involved in muscle contraction.

(iv) Connective tissues

These tissues hold and support the muscle through the component tendons and other tissues. They consist of various fibers, several different cell types and amorphous matrix. The amorphous substances include non-structured mixture of carbohydrates, proteins and lipids. The fibrous proteins are collagen and elastin. Collagen is the most abundant of all proteins in higher vertebrates making up one–third or more of the total body proteins. It is abundant in tendons, skin, bone, vascular

system of animals and connective tissues sheaths surrounding muscle. It contributes to toughness and its partially denatured product (gelatin) is a constituent in many food products. It contains one-third glycine and one-fourth proline and hydroxyproline. From nutrition point of view, collagen is not important. Moreover, elastin, is another principal protein found in connective tissues, which resist changes during heating and is low in muscles.

2.4. Nutritional value of meat

Meat provides an abundance of nutrients, generally at higher concentration than most other foods relative to calorie content. Most of the essential nutrients are present in muscle foods (Grey and Dugan, 1975). It is an excellent source of high quality protein, B-complex vitamins and certain minerals specially iron (Dutson and Lawrie,1974). From the stand-point of nutrition, the nitrogenous components of meat are probably most important, which can be divided into protein and non-protein nitrogen (NPN). NPN exists chiefly as amino acids and amide. The non-protein nitrogen fraction comprises only a small proportion of total nitrogen in meat and for practical purposes is not normally reported in the analysis Proteins are polypeptides or combinations of amino acids linked together into chain by the reaction of amino and carboxyl group of adjoining amino acids by means of peptide linkage. Proteins, in common with fats and carbohydrates contain carbon, hydrogen, oxygen. However, they also contain a large and fairly constant proportion of nitrogen.

The fat soluble vitamins present in meat include vitamins A, D, E and K while water soluble vitamins include B-complex and vitamin C. The vitamin content of meat is quite variable, being dependent on the species and age of the animal, the degree of fatness and the type of feed furnished to the animals. For instance, the pork meat contains 5 to 10 times more B-complex vitamin than that in beef or lamb. The B-complex vitamins viz. thiamine, riboflavin, niacin, pentothionic acid, vitamin B-6,

folic acid, biotin and vitamin B-12 are all found in meat and meat products. Meat and its products contribute substantial amount of B-vitamins towards meeting the dietary requirement of human. For meats with a covering of external fat layer of about 1 cm thickness, proximate composition and energy values are proteins (17%), fat (20%), moisture (62%), ash (1%) and calories (250cal) per 100g (Monin, 1998).

However, in some instances toxin components are also present in meat. These toxic components may come from naturally occurring substances in meat and meat foods, such as microbial agents, pesticides residues, food additives and substances produced during processing. Some of the components naturally present in meat, in certain cases, may be potentially harmful to human. These include cholesterol and the carcinogens created by certain cooking procedures from the lipids in the meat and meat products. In addition, there are several pathogenic and toxigenic microorganisms, which can grow on meat products.

2.5. Post–mortem changes in meat

When an animal dies, the circulatory system ceases to work resulting in lack of oxygen. The skeletal muscles stiffen in rigor mortis and remain in this condition for a period after which they soften and become flexible again. Rigor mortis is important in meat products since muscle cooked while still in rigor are much tougher than if it is allowed to pass it before cooking. The speed with which rigor develops and the length of time it persists is variable. It was earlier widely believed that the onset of rigor is speeded up by high temperature and delayed by low ones. However, it was later noted that the temperature of the carcass is of little importance compared to other factors. (Bate–Smith, 1948)

The stiffness that develops when muscle pass into rigor is the result of changes in the proteins. Living muscle fibers contain protein in a soft, pliable gel form. During rigor this gel stiffens, but when rigor passes, the muscle again become soft and

paliable. Finally, during cooking, another change called *rigor caloris* occurs and the proteins stiffen again .The changes in the protein of muscle are still incompletely understood.

When an animal is killed and the circulation of blood ceases, the degradation of glycogen continues, small amounts of intermediates accumulate, influx of oxygen stopped and lactic acid is produced. Muscle contains some buffers that neutralize the lactic acid. However, as more and more lactic acid is formed, the pH of the tissues decreases gradually. Indeed, the amount of glycogen stored in the muscle at the moment of death controls the amount of lactic acid formed and, consequently the pH of meat.

The series of chemical reactions occurring after slaughter, produces enough heat to cause a rise in the temperature of the meat. The average body temperature is 99.7°F in cattle, but shortly after death the internal temperature may rise to 103°F. The fresh meat cools very slowly even in a refrigerator because of the continuing production of heat.

Both, creatine phosphate and adenosine triphosphate (ATP) are hydrolyzed as rigor begins to develop. The muscle phosphatases capable of hydrolyzing ATP to inorganic phosphate and adenosine diphosphate, become more active as the pH drops to 6.5 and below. The ATP is then further decomposed to ribose, phosphate, ammonia and hypoxanthine (from the adenine). Contraction is no longer possible in the muscle and tension develops, which is incapable of relaxation until rigor passes.

The sarcolemma, and membrane around the muscle cell, show decrease in electrical resistance and free diffusion of ions after slaughter. The change in the semipermeable membrane indicates that not only is the protein of the muscle fiber changing but the substances that make up the membrane, the sarcolemma, may also change chemically.

The ultimate pH attained by the meat has important effects on other properties of the meat. Meat with a high pH is darker in colour than normal, it is slimy to touch and does not allow salt to penetrate readily. At high pH, the swelling of the protein is greater and because of this the ability of oxygen to diffuse through the tissue decreases, as a result the proportion of oxymyoglobin to myoglobin changes and gives darker colour.

The post-mortem effects bring about changes in the quality attributes of meat viz. texture, water holding capacity, flavour, etc without affecting the nutritional quality. After the passing of rigor mortis, meat becomes progressively more tender, juicier and more flavorsome. The speed with which this ripening or aging occurs depends on the time and temperature at which the carcass is kept. Changes occur quite rapidly at room temperature but more slowly at lower temperatures. Poorly finished carcasses rapidly develop off-flavour and putrify. Aging of beef has been investigated and discussed by Lowe (1955) however, the numerous changes that occur on ripening are still not fully known. The reactions are the result of autolytic action in the cells that fragment macro molecules through the action of enzymes and changes the cell conditions, particularly the pH.

The content and distribution of nitrogen compounds and particularly the aminoacids in raw and cooked beef and in the dripping from the meat before and after aging /ripening had been determined by Ginger et al. (1954). Aging caused an increase in the free amino acids nitrogen .The nitrogen in dripping of freshly slaughtered meat is mostly non-protein nitrogen (NPN) with a large proportion of it as amino nitrogen. Upon aging for two weeks, the dripping exhibits an increase in the amount of NPN. Extensive studies on the changes in meat due to ripening have been reported (Solov and Piluskaya,1951; Sizova, 1956 ; Drozdov, 1955)

2.6 Meat microbiology

Meat animals may be regarded as a source of edible tissue sandwiched between two regions that are heavily contaminated with microorganisms. These two regions are the external layers of skin, hair, wool or feathers and the internal intestinal tract and its contents. The first aim of the abattoir is to harvest the edible tissue (meat) from these highly contaminated layers with as little contamination as possible. Second, care is needed to ensure that contamination of dressed carcasses and edible offal from sources within the abattoir itself is kept to a minimum. Subsequent procedures for the handling of meat through chilling, freezing, cooking, packaging, and distribution to the consumer are aimed at reducing or preventing increases in the microbial content that may occur either by growth or by further contamination.

2.6.1. Source of microbial contaminations

(i) Non-sterile knife used for exsanguinations.

(ii) Head,legs,hide, offal removal during slaughter.

(iii) Water source for cleaning.

(iv) Surface contacts during storage.

(v) Contacts during fabrications.

(vi) Contacts during handling and processing.

Majors sources of meat contamination are hide, feed, manure, viscerae, equipments, clothing and hands of personnel, air, water, walls, floor and doors, etc. These points affect initial microbial load which is the major factor in determining the shelf life of meat.

2.6.2. Microorganisms present in meat

The three major types of microorganisms in meat includes bacteria, yeasts and mold. The relative importance of the wide variety of microorganisms that may contaminate meat during its production is dependent on the type of microorganisms

and the subsequent treatment of the meat. In this reference, Tables 2.2 and 2.3 show the average number and types of microorganisms contaminating beef in packaging plant's slaughter room and the frequently isolated microorganisms from meat.

2.6.3. Factors affecting microbial activity

There are two types of factors, extrinsic and intrinsic, which affect the microbial activity, as specified below:-

(i) Extrinsic factors

The extrinsic factors are temperature, relative humidity, oxygen concentration and physical state as described below:

(a) Temperature

It is one of the most important factor that effects the type, rate and extent of microbial growth (Haines, 1934 and 1937). In case of psychrophiles, optimum growth occurs at temperatures lower than 20°C, and these microorganisms are able to grow at normal refrigeration temperatures (Eddy,1960). Molds are more *psychrophilic* than yeast and bacteria. Psychrotropic, *Pseudomonads* are able to grow down to -3°C (Barnes, 1976) and some *psychrotropic* molds down to about –5°C to –7°C (Barnes 1976 ; Gill and Lowry 1982). Thus *psychrophiles* are able to grow at refrigeration temperature but have an optimum temperature for growth at 15°C or less and a maximum temperature for growth at about 20°C (Morita, 1975). Mesophilic microorganisms are not able to sustain growth below about 5 to 7°C and grow optimally at 30°C to 40°C. The rate of growth decreases with the decrease in temperature from optimum value, which varies for different microorganisms. *Thermophiles* have higher minimum and optimum temperatures for growth. They fail to grow below 35°C to 40°C are being able to grow upto 80°C and above. Facultative *thermophiles*, or thermotrophs, have minimum and optimum growth temperatures that fall between those for *mesophiles* and *thermophiles* (Mossel, 1977). The distinction

Table 2.2 Average number of microorganisms contaminating beef in packing plant's slaughter room

Sample	Bacteria	Yeast	Mold
Beef dressed on floors	6,400830,000/cm^2	050,000/g	120,000/g
Soil from animals (dry)	00110,000,00/g	200,000/g	060,000/g
Animal feces (fresh)	0090,000,000/g	180,000/g	001,600/g
Rumen content	2,000,000,000/g		000002/cm^2

Adapted from: Empey and Scott (1939)

Table 2.3 Commonly present meat micro flora

Product	Microorganisms Isolated
Fresh and refrigerated meat	Bacteria *Acinotobacter, Moraxella, Pseudomonas, Aeromonas, Alcaligenes and Micrococcus* Mold *Cladosporium,Geotrichum, Sporotrichum, Mucor and Thamnidium* Yeast *Candida, Torulopsis, Debaryomyces and Rhodotorula*
Processed and cured meat	Bacteria *Bacillus Micrococcus, Serratia, and Staphylococcus.* Mold *Aspergillus, Pencillium, Rhizopus, and Thamnidium.* Yeast *Debaryomyces, Torula, Torulopsis, Trichosporon,and Candida*

Adapted from: William and Dennis (1958)

between these groups is not sharp since there is a gradual merging of the minimum, optimum, and maximum temperatures for growth of different microorganisms.

(b) Relative humidity / humidity

When relative humidity (RH) is too high, moisture condenses on meat and makes it conducive to microbial growth. On the other hand, if the RH is too low, the meat looses moisture to the atmosphere resulting in reduced yield and dehydration of meat, which limits the microbial growth. The drying process also inhibits the bacterial growth.

The major cause of spoilage of finished dry cured meat product is due to yeast and mold growth during storage under conditions of high humidity (Tompkin, 1986). The drying conditions with less than 75% RH do not afford desirable mold growth (Bacus,1986). The european style sausages are fermented for 3 to 4 days at 15-24°C with 80-90% relative humidity. Studies on sausage production and storage revealed that temperatures in excess of 30°C and high humidity (87%) favour aflatoxins production (Alvarez and Barrera et al., 1982). Nevertheless, humidity plays an important role in production of fermented or aged meats.

(c) Oxygen requirement

The oxidation-reduction potential and oxygen tension are the important factors promoting growth of surface spoilage microorganisms on meat and meat products. The microorganisms on the basis of the oxygen tension have been categorized as aerobes, anaerobes and facultative anaerobes.

Aerobic microorganisms (e.g. *Pseudomonads*) couple the oxidation of a substrate to the reduction of oxygen by means of a respiratory chain containing cytochromes. The flow of electrons through the respiratory chain to oxygen results in the formation of ATP from ADP and phosphate. ATP provides energy for growth and metabolic processes. Some anaerobic organisms are also able to synthesize a modified

respiratory chain that is able to use nitrate (or nitrite) instead of oxygen as the terminal electron acceptor

Microaerophilic organisms are aerobes, which grow best at oxygen-concentration less than found in air (e.g., *Campylobacter jejuni (coli)*). The concentration of oxygen in air may be bacteriostatic or bactericidal for such organisms. Obligate anaerobic organisms are not able to synthesize the component of oxygen (or nitrite/nitrate) linked respiratory chain. These organisms, obtain energy for growth by fermentation in which a variety of organic compounds, carbon dioxide, hydrogen, and sulphur compound are involved in oxidation reduction reactions. Facultative anaerobes (e.g.*Enterobacteriaceae*) are able to grow aerobically using respiratory chain coupled oxygen and anaerobically using fermentation reactions for generation of energy.

(d) Physical state

The relative importance of the wide variety of microorganisms that may contaminate meat during its production is dependent on the types of microorganisms and the subsequent treatment of the meat. Microbial load increases because of larger area of exposed surface, more readily available water nutrients and great oxygen penetration and availability. Contacts with greater number of surfaces, grinder, belts, etc., result in higher microbial loads.

(ii) Intrinsic factors

The intrinsic factors responsible for microbial activity in meat products are moisture-content, pH, oxidation-reduction potential, nutrient availability, etc. as described below:

(a) Moisture and osmotic pressure

The availability of moisture is the most important requirement for microbial growth on meat. The availability of moisture is complimentary to that of osmotic

pressure, which is a function of the concentration of soluble, dialyzable substances. in the aqueous medium. High solute concentration tends to inhibit growth and desiccation of the substrates restricts microbial growth on frozen meat products (Lawrie, 1991).

The significance of water with respect to meat spoilage have been studied in detail by Scott (1936 ; 1957 ; 1962) who also coined the term water activity (a_w) in this context. The water activity (a_w) of a solution is the ratio of its vapour pressure to that of pure water at the same temperature. It is inversely proportional to the number of solute molecules present. In general, molds and yeasts tolerate higher osmotic pressures than bacteria (Haines, 1937). Bacteria grow from an a_w of just under 1.0 down to an a_w of 0.75, where as the yeasts and molds grow slowly at an a_w 0.62 (Scott, 1957). Scott (1936) showed that decreasing the a_w decreased the growth rates of yeasts, mold and bacteria on meat surfaces (Fig 2.3).

The bacteria responsible for meat spoilage have a_w between 0.98 and 0.96 (Leistner et al., 1981). These a_w levels are attained in meat between –2°C and –4°C (Table 2.4). The optimum a_w for several food poisoning strains of *S.aureus* has been shown to about 0.995 (Scott, 1953). At 38°C, the level of a_w at which this organisms would grow on dried meat has been reported as 0.88. A similar study of the water requirements of *Salmonellae* has been carried out by Christian and Scott (1953).

The nature of the solute determining a_w is not without effect, as the microorganisms can tolerate a greater reduction of a_w in solutions of glycerol or other polyols than in solutions of sugars or electrolytes (Brown,1976). Such specific solute effects are, however, largely irrelevant to consideration of the effects of reduction of a_w in fresh meats, as some solutes of quantitative importance are always present. The use of a_w has been challenged (Franks, 1982,1991) when applied to non-equilibrium systems (most food products) and because microbial response is more dependent on

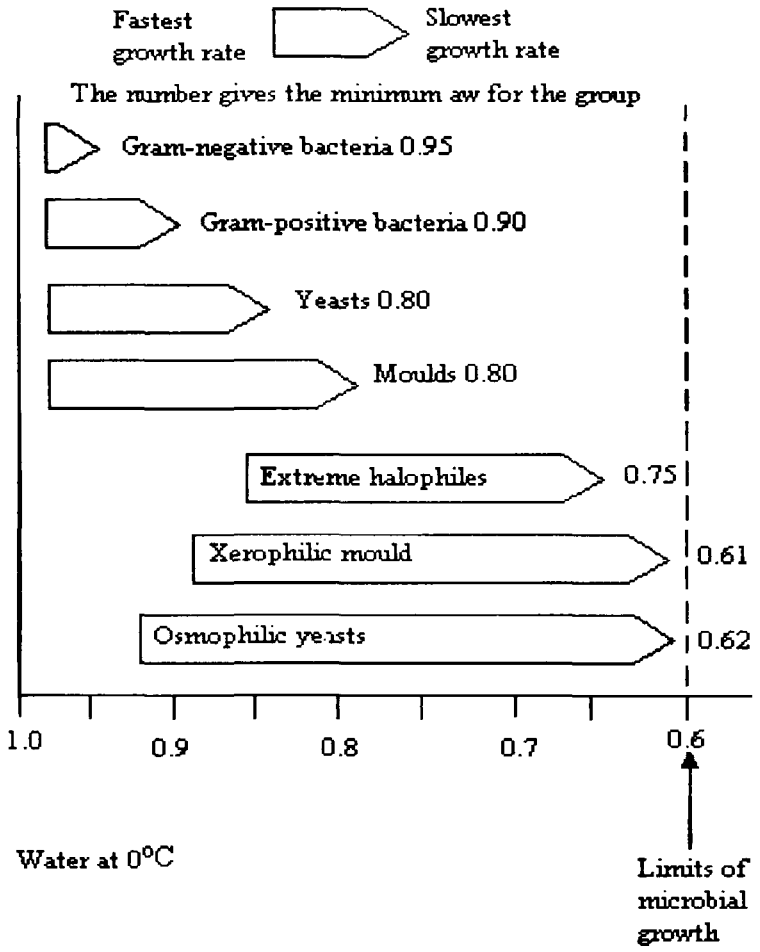


Fig 2.3. Water activity ranges for microorganisms found in foods
Adapted from: Garbutt (1997)

Table 2.4 Water activity (a_w) of meat at various freezing temperatures.

Temperature (°C)	Fresh meat
25	0.993
	unchanged ↓
0	
-1	
-2	0.981
-3	0.971
-4	0.962
-5	0.953
-10	0.907
-15	0.864
-20	0.823

Adapted from: Rodel and Krispien, (1977)

the solute used to reduce a_w (Ballesteros et al., 1993 ; Chirife and Buera, 1994) than on a_w per se. Nevertheless, the water activity (a_w) is still a very valuable empirical parameter for microbial stability if equilibrium conditions are assumed in the experimental time frame (e.g. shelf life) (Chirife and Buera, 1996).

Different theories have been proposed for a more reliable indicator, than a_w for microbial stability in foods. "Mobility" has been addressed as a food stability indicator (Duck–Worth, 1981; Slade and Levine, 1987;Vandenberg and Bruien,1981). It has been suggested that the glass transition temperature (Tg), a parameter describing system mobility, closely correlates to microbial response (Slade and Levine, 1987). Although, this concept is very promising and innovative, the experimental evidence did not support a correlation between Tg and microbial activity (Chirife and Buera, 1996; Buera et al., 1998; Vittadini et al., 2003). The Tg theory considers mobility on a structural and macromolecular level and therefore, it is a parameter descriptive of the physical state of macromolecules. This however, differs from the molecular mobility of smaller molecules such as water, which has been considered the single most important factor governing microbial response.

'Mobile water' correlates with germination time of mold spores better than a_w (Lang; 1980, Kou and Chinachoti, 1992 ; Chinachoti, 1993; Pham et al., 1999). Also, the *Staphylococcus aureus* growth reportedly correlates better with the amount of mobile water than with a_w of the culture media (Lawrie et al., 1998).

(b) pH

The post mortem pH of meat is determined by the amount of lactic acid produced from glycogen during anaerobic glycolysis. Amount of lactic acid will be low if the glycogen is depleted by fatigue, inanition or fear in the animal before slaughter. Since pH is an important determinant of microbial growth, it is obvious that the ultimate pH of meat is significant for it is resistance to spoilage

(Lawrie, 1991). Fig 2.4 shows the pH ranges and optima for the majority of bacteria, yeast and molds.

Most bacteria grow optimally at about pH 7.0 and not well below pH 4.0 or above pH 9.0. However, the pH of maximal growth is determined by the simultaneous operation of variables other than the degree of acidity or alkalinity itself. Some of the biological enzymes, which cause spoilage, may have different optima from that of the organism itself. Thus, bacterial proteolytic enzymes operate best near neutrality and the enzymes, which attack carbohydrates tend to have optima pH below 6.0. However, the organisms such as lactic acid bacteria, with the pre-dormant activity of carbohydrates break down exhibit pH optima between pH 5.5 and 6.0.

In fresh meat, a high pH, especially in the deeper areas of the carcass, promotes bacterial growth which causes 'bone taint'. The high ultimate pH in muscle is because of a deficiency of glycogen at death and also lacks the glucose, which is produced by amylosis post mortem, albeit in much smaller quantity than lactic acid by glycolysis. In the absence of readily available carbohydrate substrate, the microorganisms attack aminoacids and cause early spoilage producing off-odour and discoloration (Newton and Gill, 1978).

Table 2.5 shows the pH ranges for food pathogens. Generally, the bacteria favour neutral pH of meat. Mold grow in the wide pH range from 2.0 to 11.0, but often favour acid pH and thrive in media that are too acidic for either bacteria or yeast. Yeast can tolerate acid and grow at pH 4 .0 to 4.5.

(c) Oxidation-reduction potential

Oxidation–reduction potential (E_h) is one of the critical parameter that affects the growth of bacteria and fungi on a rich nutrient source. The ability of meat to undergo, reduction or oxidation is reflected by oxidation-reduction potential. The aerobic microbes are favoured by high oxidation-reduction potential while anaerobic

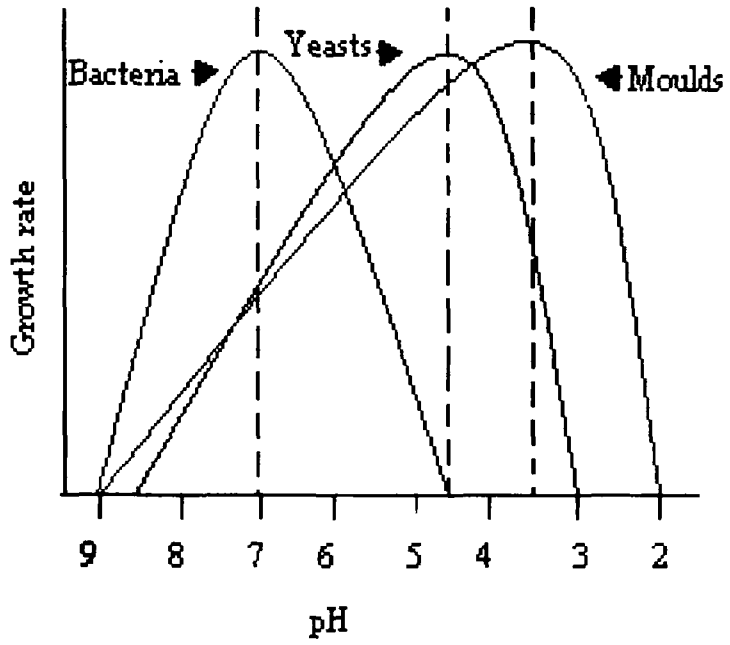


Fig 2.4 Effect of pH on the growth rate of bacteria, yeast and molds.
Adapted from: Garbutt (1997)

Table 2.5 pH ranges for food pathogens.

	pH		
Pathogens	**Minimum**	**Optimum**	**Maximum**
Bacillus cereus	4.9	7.0	9.3
Campylobacter jejuni	2.3-5.8	7.0	9.0
Clostridium botulinum	4.2	7.0	9.0
Clostridium perfringens	5.5	7.0	8.0
Escherichia coli	4.4	---	9
Lactobacillus sp.	3.0	---	7.2
Listeria monocytogenes	4.1	6.0-8.0	9.6
Molds	1.5-2.0	---	11
Salmonella spp.	4.05	7.0	9.0
S.aureus	4.0	6.0-7.0	9.8
Shigella sp.	4.5	---	8
Streptococcus lactis	4.3-4.8	---	---
Yeasts	1.5	---	8.0-8.5
Yersenia	4.6	7.0-8.0	9.0

-- : *Data* not available
Adapted from : Garbutt (1997)

microbes are favoured by low oxidation–reduction potential. Microbes are capable of altering oxidation-reduction potential of meat to the extent that activity of other organism is restricted. Anaerobes may decrease oxidation–reduction potential to such a low levels that growth of anaerobic organisms is inhibited. Jay (1978) has stated that although fungi tolerate wide variations in temperature, pH and water activity, they are dominated by bacteria in meat because of their relatively slow growth rates and high E_h requirements. The change in E_h occurs immediately after death and is probably due to the removal of the last traces of oxygen by the still surviving activities of the tissues oxygen-utilizing enzymes system.

The effect of E_h on microbial growth is to prolong the initial lag phase, (Barnes and Ingram, 1956). Once the organism become adjusted to a high E_h, the growth rate remains the same as at a low E_h. The E_h of meat not only depends on the oxygen tension but also on concentration of molecules having marked electro positive character. Thus, the presence of nitrate in cured meat probably exerts an indirect antibacterial effect through raising the E_h of the system. Under anaerobic conditions at low pH (5.5) nitrates protect bacteria against nitrite (Eddy and Ingram, 1956).

2.6.4 Microorganisms associated with meat spoilage

The major microorganisms, which grow on meat and cause spoilage are discussed as under:.

(i) *Staphylococcus*

Staphylococcus are Gram positive, facultative anaerobic cocci with a tendency to grow in clusters. They are widely distributed in nature and frequently found as part of the microbial flora of meat and poultry products. Scheleifer et al. (1984) described 23 species of practical importance on meat and poultry, however, the most common amongst all is *S.aureus* (Evans,1986). This bacterium causes severe infection on meat in animals and birds besides causing food poisoning. *S.aureus*

grows very vigorously under anaerobic conditions in a temperature range of 10°C to 45°C (Troller, 1976).

S.aureus are non-spore forming bacteria, which do not produce any heat resistant bodies. They are readily killed by heat processing or cooking temperature to which meat and poultry products are normally subjected before being consumed. These are salt tolerant and can grow in medium containing upto 12% salt. Most selective media for *Staphylococci* contain 7.5% salt. It has been reported that 1 to 10% salt in medium reduces the rate of toxin production (Pereira et al., 1982) *S.aureus* is also capable of growing over a wide pH range. Its growth can occur under alkaline conditions as high as pH 9.6 and under acidic conditions as low as pH 5.0. However, at pH of 6.0 and below the growth rate decreases and the possibility of producing the enterotoxin also reduces substantially (Troller, 1976; Pereira et al., 1982).

Staphylococci are capable of fermenting a fairly wide range of carbohydrates. Under anaerobic conditions, *S.aureus* carry out a homolactic fermentation of glucose with more than 90% of sugar being converted to lactic acid via the conventional glycolytic pathway. Under aerobic conditions, they metabolize the glucose to CO_2 and water via the normal respiratory pathway involving the tricarboxylic acid cycle and a cytochrome–linked pathway of electron transport. They are also capable of carrying out limited transformations of a wide range of nitrogenous compounds. Most strains hydrolyze native animal's protein but do not cause proteolytic spoilage of meat and poultry products (Evans, 1986).

S.aureus is predominantly present on the skin and nasal membranes, in the intestinal tract. It causes variety of cutaneous infections in the human population, meat animals, and poultry. Therefore this microorganism, constitute part of the bacterial flora prevalent in meat and poultry processing facilities and are part of the

flora on the products, although normally as a minor component (Evans, 1986). Fresh meat and poultry, may also serve as source of these organisms for other foods where they may proliferate. Particularly, the cured meats are more vulnerable. *S.aureus* have been detected in 73% of the various food samples tested, which were milk, and meat based, bakery food, seafoods and some miscellaneous foods. (Bacus, 1986).

(ii) *Salmonella*

Salmonella are commonly present on raw meat and poultry (Silliker and Gabis, 1986).The incidence and numbers of *Salmonella* in meat and poultry, however, varies with the species of animal from which it is derived, the geographical location, pre-slaughter holding conditions, processing conditions and other factors. *Salmonella* are pathogenic to man and animals. There is a direct link between the occurrence of *Salmonella* in living animals, on the meat derived from them, and human '*Salmonellosis.*' On a world wide basis, the epidemiological records for food borne diseases prove that meat and poultry are the vehicles responsible for most out-break of this disease (Hobbs, 1974).

The optimum temperature for growth of *Salmonella* is 35-37°C. Environmental factors including the substrate, pH, water activity and competing microflora influence its growth rate. Tables 2.6 and 2.7 represent the growth and generation time of *Salmonella* in variety of foods and at varying temperatures, respectively (Mackey et al., 1980). The growth may also occur at refrigeration, however, at exceedingly slow rate.

Salmonella growth has been reported in liquid medium at pH values as low as 4.0 (Chung and Goepfert, 1970), but the optimum pH is generally accepted to fall between 6.5 and 7.5 (Silliker and Gabis, 1986). The minimum a_w (water activity) required for growth of *Salmonella* species in laboratory media has been determined to be 0.941 while their maximum growth rates were obtained at 0.995 a_w (Christian and

Table: 2.6 Effect of temperature on the growth of *Salmonella* in food.

Food	Growth (°C)	No growth (°C)	References
Beef and pork	----	4.4-10	Gomutputra and Fabian (1953)
Chicken a la King	6.7	5.6	Angelotti et al. (1961)
Ham Salad	----	10.0	Angelotti et al. (1961)
Cream Custard	----	10.0	Angelotti et al. (1961)
Crab meat	----	5.0	Berry, (1964)
Crab meat (sterile)	8.0	5.0	Mathches and Liston (1968)
Sole	8.0	6.0	Mathches and Liston (1968)
Egg	10.0	2.0	Ayres and Taylor (1956)
Egg	10.0	5.0	Stokes et al. (1956)
Liquid egg	11.1	7.2	Gibbons et al.(1944)
Ground pork	10.0	4.0	Alford and Palumbo.(1969)
Ground pork	----	7.0	Sulzbacher (1952)
Ground beef	12.5	7.0	Goepfert and Kim (1975)
Ground beef	----	5.0	Tiwari and Maxey (1972)
Luncheon meat	----	5.0	Goepfert and Chung (1970B)
Bacon	16.0	5.0	Farrell and Upton (1978)
Ground beef (vacuum-packed)	12.0	5.0	Davidson and Witty (1977)
Sterile beef	8.0	7.0	Shaw and Nicol (1969)

Adapted from: Mackey et al. (1980)

Table 2.7 Variability in growth rate of *Salmonella* as a function of temperature in different media

Temp (°C)	Organism	Substrate	Generation Time (hr)	References
8.0	*S.heidelberg*	Broth +1% NaCl	19	Matches and Liston (1972)
	S.heidelberg	English sole	28	Matches and Liston (1972)
	S.heidelberg	Sterile crab	31	Matches and Liston (1972)
10.0	*S.oranienburg*	Sterile beef	35	Shaw and Nicol (1969)
	S.oranienburg	Ground pork	9.4	Shaw and Nicol (1969)
	S.derby	Ground pork	19	Alford and Palumbo (1969
	S.enteritidis	Ground pork	22	Alford and Palumbo (1969
	S.thompson	Ground pork	17	Alford and Palumbo (1969
	Mixture of Strains	Beef surface	8-26	Mackey et al. (1980)
12.0	*S.heidelberg*	Broth	12	Matches and Liston (1972)
	S.typhymurium	Broth	12	---
	S.derby	Broth	12	---
12.5	Mixture of Strains	Beef surface	5.2-10.2	Mackey et.al . (1980)
15.0	Mixture of Strains	Beef surface	2.9- 03.5	Mackey et.al . (1980)
	S.oranienburg		4.6	Shaw and Nicol (1969)

---: Reference is not available

Adapted from: Pearson and Duston (1986)

Scott, 1953). The water activity of red meat and poultry is near optimum for growth of *Salmonella*. On the contrary, the salt in cured meats reduces the a_w,, which results in reduced growth rate. Altogether, the incubation temperature, competing microflora and pH combine with a_w influence the growth of *Salmonella*. Chung and Goepfert (1970) reported that *Salmonella* rapidly die in the presence of a lactic acid bacteria starter culture during the 24 h fermentation of sausage emulsion at 30°C. Moreover, if salt is omitted from the sausage emulsion, *S.typhimurium* multiply with in 12 h incubation at 30 to 37°C, suggesting the influences of a_w (Smith et al., 1975).

Salmonella are heat sensitive and ordinary pasteurizing or cooking conditions are generally sufficient to kill cells in high moisture foods. The heat resistance of *Salmonella* is markedly increased as a_w decreases (Silliker and Gabis, 1986). Extensive studies on the heat resistance of *Salmonella* has been reported (Genigeorgis and Riemann, 1979).

There is an epidemiological evidence that storage of meat and poultry increases the *Salmonella* hazard. Modified atmosphere may provide some protection against the growth of *Salmonella* in red meats (Silliker and Wolfe, 1980 ; Luiten et al., 1982). Bajaj et al. (2003) studied the incidence of *Salmonella* in meat and poultry and growth inhibition of *Salmonella enteritidis* by organic acids viz. propionic acid, lactic acid, acetic acid, citric acids, etc. The growth inhibitory activity of organic acids against *Salmonella enteritidis* has been found to be in order as propionic acid < acetic acid < lactic acid < citric acid. About 82 isolates of *Salmonella enteritidis* from humans, chicken and egg have been characterized based on their antibiotic resistance pattern, plasmids profile, phagetyping (PT), outer membrane proteins (OMP) and lippolysaccharides (LPS) pattern (Icgen et al., 2002). Del Cerro et al. (2003) have described the usefulness of selected PCR protocol for the detection of *Salmonella* in

117 samples of animal origin (17 raw minced meat, 27 raw chickens meat and raw sausages, 25 egg samples as well as 18 poultry faecal samples.

(iii)*Yersinia*

Animals are important reservoirs of *Yersinia*. *Yersinia pseudotuberculosis* has been found in the digestive tract and faeces of a wide variety of animals and birds. Capita et al. (2002) reported the presence of *Yersinia* spp. *Yersinia enterocolitica* and *Y. frederiksenii* in poultry meat. *Yersinia* reach raw meat and poultry from intestinal and oral cavity contents, hides, feathers and processing equipment. Cooking meat and poultry to internal temperatures of 60°C or above for a few minutes should kill these organisms unless the level of contamination is very high. Efforts to prevent cross contamination from raw poultry to cooked foods are essential aspects of a control programme The recommended temperature (7°C) that prevent growth of other pathogenic food-borne bacteria, unfortunately do not prevent the growth of *Y. enterocolitica*, although lag period prolongs. (Bryan, 1986). However, freezing adversely affects the survival of *Y.enterocolitica* in meat. Significant reduction inviability of this organism occur when roasted beef has been stored at -23°C for 28 days (Hanna et al., 1977). In ground beef, the survival of these organisms declined by 83% in 30 days when stored at -20°C (Zawahry and Rowley, 1979). In ham stored for 7 days at –10°C, almost a 2 $\log^{10}$ decrease of *Yersinia enterocolitica* has been observed Asakawa et al. (1979) Leistner et al. (1975) also observed a decrease in numbers of these organisms in chicken stored at -18°C for 90 days.

(iv) *Listeria monocytogenes*

Listeria monocytogenes is a gram positive, non-sporulating food borne pathogen. It is ubiquitous in nature and therefore its contamination occurs in a variety of foods, such as raw meat, milk and milk products, sea foods and vegetables, (Farber and Peterkin, 1991; Pini and Gilbert, 1988; Bhilegaonkar et al., 1997). Consumption

of contaminated foods can cause severe and often fatal infections in the susceptible hosts. Therefore, this organism has been of major concern in the food industry in recent years. A routine analysis of food samples for detection of *L.monocytogenes* is an essential step in determining the quality of food. Dykes (2002) studied the behaviour of *L.monocytogenes* on two processed meat products (bologna and summer sausage) as influenced by temperature or attached growth during pre-incubation.

Stekelenburg (2003) studied the enhanced inhibition of *L.monocytogenes* in Frank furter sausage by the addition of potassium lactate and sodium diacetate mixtures. Comparison of *Lactobacillus* and *L.monocytogenes* growth and the sensory qualities with a reference product revealed that addition of 2-3% solution, consisting of 56% potassium lactate and 4% sodium diacetate to Frankfurter-type sausage inhibit the development of *L.monocytogenes* during storage at 4°C.

Spices have also been reported to exhibit suppressing action on *L.monocytogenes* and other food borne pathogens (Bahk et al., 1990; Aureli et al., 1992; Lis-Balchin and Deans, 1997; Hao et al., 1998; Smith-Palmer and Stewart., 1998). Cinnamon powder, when examined for anti-listeric activity in meat and cheese exhibited bacteriostatic action on *L.monocytogenes* in both the foods (Menon et al., 2002). Foods treated with 6% cinnamon showed 1-2 $\log_{10}$ less *Listeria* count per gram food stored at 30°C for 7 days and at 7°C for 15 days. Treatment with 3% cinnamon also slowed down the growth of the microorganisms significantly in meat. The activity of 3% cinnamon, however, was not appreciable in meat at 7°C. Earlier, Bahk et al. (1990) reported 0.5% cinnamon powder to be effective against *L.monocytogenes* in culture medium while Aureli et al. (1992), Lis-Balchin and Deans (1997) Smith Palmer and Stewart (1998) reported oil of cinnamon to be anti-listeric.

Inactivation of acid and non adapted *L.monocytogenes* on beef slices has been studied during drying and storage of jerky, formulated with modified marinades

(Calicioglu et al., 2002). The data revealed that the bacterial population dropped below the detection limits (0.4 log CFU cm^{-2}) with in 4h during drying or remained undetectable even after 60 days of storage depending on acid adoption, pre-drying treatments and agar media.

(v) *Escherichia coli*

Escherichia coli resides in the lower part of the intestinal tract of warm blooded animals. It is the most common oxygen tolerant bacterium in the large bowels of human beings. Most strains of *E.coli* are harmless commensals, but pathogenic strains can cause enteric illness either by elaborating a cholera like enterotoxin or by penetrating the intestinal epithelium as Shigella does (Bryan et al., 1979; Sack, 1975), Because of the utriquitous nature of *E.coli,* it commonly reaches the carcasses and cuts of meat during slaughtering and processing. These organisms can multiply on equipments surfaces, so their presence on meat may not indicate direct fecal pollution.

There are numerous reports of *E.coli* on meat during slaughtering and processing. For instance, it has been reported in ground beef (Geopfert, and Hicks, 1976 ; Reis et al., 1980, Tamminga et al., 1982), sausage (Reis et al., 1980) and on poultry (Kim and Stephens, 1972). Only a part of the *E.coli* flora on foods is pathogenic. Of 240 *E.coli* isolates from foods, only 19 (8%) were enterotoxigenic (Sack et al., 1977). Toxigenic isolates were found on 10% of sausages, 7.5% of ham burger, and 5% of keebe (Reis et al.,1980). *E.coli* is commonly found on the hands of workers in the food industry (Harwood and Minch, 1951) therefore, thorough cooking of meat foods is an important control measure. Outbreaks can often be prevented by storing cooked foods in a manner that prevents multiplication of *E.coli.*

E.coli 0157:H7 is recognized as a food-borne pathogen of primary concern. It causes hemorrhagic colitis, haemolytic uremic syndrome and thrombo cytopenic purpura (Carter et al., 1987 ; Swerdlow et al., 1992 ; Tarr, 1994). *E.coli* is implicated

in outbreaks caused by consumption of inadequately cooked contaminated beef (Riley, 1987; Belongia et al., 1991; Doyle, 1991; Armstrong et al., 1996), and Turkey roll (Rayan et al., 1986; Carter et al., 1987) besides other foods like mayonnaise (Weagant et al., 1994; Raghubeer et al., 1995), raw milk (Borezk et al., 1987), unconfirmed food vehicles (Watanabe et al., 1996), etc. Asymptomatic cattles are the primary reservoir of this pathogen (Cray and Moon, 1995 ; Zhao et al., 1995). With an increasing incidence of food borne illness associated with *E.coli* O157:H7, its elimination from ground beef has become an important area of research. The center for disease control and prevention in USA had estimated that food borne diseases caused by *E.coli* O157:H7 accounted for 62,438 cases of illness, 1843 hospitalization and 42 death in US each year (Mead et al., 1999).

It has been demonstrated that this pathogen was able to tolerate acidic environments in a range of fermented and acidified products such as processed "Salami" (Clavero and Beuchat,1996) during the processing of fermented meat. (Glass et al., 1992 ; Faidth et al., 1998 ; Riordon et al., 1998) and in ground roasted beef stored at 5°C for three days (Abdul Raouf et al., 1993). The heat resistance of this organisms has been studied extensively in meat (Doyle and Schoeni, 1984 ; Line et al.,1991 ; Ahmad and Conner, 1995 ; Ahmad et al., 1995 ; Jakson et al., 1996 ; Kotrola and Conner, 1997 ; Juneja et al., 1997). Juneja and Marner (1999) developed a thermal inactivation model for *E.coli* O157:H7 with temperature, pH, NaCl (salt) and sodium pyrophosphate as controlling factors. Kaur et al. (1998) studied the effects of a variety of factors including growth phase, growth temperature, heat shock, variable heating rate, nature of heating medium, polyphosphate and water activity on the heat resistance of *E.coli* O157:H7. Juneja and Novak (2003) also investigated the heat resistance of *E.coli O157:H7* in cook in-bag ground beef as affected by pH and acidulant. Regardless of the acidulant used to modify the pH, the values at all

temperatures were significantly lower ($P<0.05$) in ground beef at pH 4.5 as compared with beef at pH 5.5. At the same pH levels acetic acid rendered *E.coli* O157:H7 more sensitive to the lethal effect of heat

(vi) Yeasts and molds

Yeasts are generally thought to be of little importance for meat spoilage but mold growth causes considerable loss. At temperatures above the freezing point of meat, spoilage by the relatively rapid growth of bacteria usually precludes spoilage by the slower growing yeast and mold. Meat is spoiled by fungi only when it is held under conditions that inhibit bacterial growth (Gill, 1986). There are 50,000 to 200,000 species of fungi (Kobayashi, 1980). Fungal counts in fresh beef and poultry range typically between 10^2 and 10^5 per gram (Jay, 1979). However, in some cases molds are believed to give quality attributes to products such as in fermented meats and aged beef.

Jay (1978) reported that only about 20 mold genera had been isolated from meat. Amongst all the *Aspergillus* and *Penicillia* are the most common. Other molds associated with meat spoilage were *Thamnidium*, *Mucor*, *Rhizopus*, *Cladosporium* and *Sporotrichum*. A survey of the incidence of yeast in meat products revelead that the species *Debaromyces, Candida*, *Toroulopsis* and *Rhodotorula* were most frequently isolated (Jay,1978).

Mold growth causes considerable meat spoilage. It is usually considered that mold spoilage is the result of growth of these organisms on frozen meat when bacterial growth is inhibited by the low storage temperature. Minimum growth temperature for molds varies from -10°C to -12°C, or even -18°C (Ingram and Mackey 1976 ; Leistner et al., 1981; Gracey 1981).

Gill and Lowry (1982) Lowry and Gill (1984) have demonstrated the abilities of molds to grow on frozen meat. Most meat spoiling molds are moderately

and have intrinsic minimum growth temperatures on unfrozen media of about -5°C or above. Temperature of -5°C seems to be the practical minimum temperature for growth of molds on meat (Gill and Lowry, 1982 ; Lowry and Gill 1984). Molds grow very slowly at -5°C, however, when meat or poultry is held at this temperature for long periods, a flora of psychrotropic yeasts develops, attaining the cell density of 10^6 /cm^2 after 4 months. The yeasts grow as discreet, ultimately visible, colonies on the dry environment provided by the frozen meat surface at -5°C. Visible mold colonies do not appear until about the eighth month of storage (Schmidt-Lorenz and Gutschmidt, 1969; Lowry and Gill, 1984).

Gill (1986) also reported that mold spoilage develops on frozen meat only if it is held for prolonged periods at 2°C or 3°C below its freezing point. If the meat surface reaches higher temperatures, mold spoilage can occur, if surface drying inhibits bacterial growth. The latter circumstances are likely to be the useful conditions leading to mold spoilage during frozen storage. Thus, yeasts are the organisms best able to grow on meat that remains frozen at the surface.

Growth of yeast does not seem to impart undesirable odour or flavour to the meat, and the pin-head colonies formed at freezing temperatures might easily by overlooked, particularly on flat surfaces (Winger and Lowry, 1983). However, if the meat is then exposed to higher temperatures, the preformed yeast colonies on it would rapidly increases in size to give more visible spoilage. Visible spoilage may be further enhanced by deposition of heme pigments resulting in the appearances of brown spots on fat surfaces (Egan and Shay, 1977).

The major cause of spoilage of finished products is due to yeasts and mold growth during storage in conditions of high humidity (Tompkin, 1986). Fermented meat products rely upon controlled microbial activity of specific types of molds and bacteria and sometimes even yeasts. Yeasts like, *Debaromyces kloeckeri*, *D.hansenii*,

D.cantarelli D.pfaffia Debaromyces spp and mold like *Penicillium expansum, P. moczynski, P. simplicissimum, P.nalgiovensis, P.candidum, P.camembert, P.comne, P.roqueforti, Penicillium sp . Scopulariopsis, brevicaulis* and spp. etc. have been species described as meat starter cultures along with several bacteria (Bacus,1986).

2.7. Meat spoilage

The term spoilage with reference to meat signifies any single symptom or group of symptoms of overt microbial activity, manifested by change in meat odour, flavour or appearance (Gill, 1986).

As meat comprises of various proportions of muscle, fat and connective tissues. The fat and connective tissues are very different than muscle, therefore, spoilage of muscle tissues, under most circumstances, is the critical factor in determining the shelf life of meat. The quantities of the bulk materials, fats and protein do not alter during rigor development in meat also do not serve as substrates for microbial attack before the development of spoilage (Dainty et al., 1975; Gill and Newton, 1980). Instead, bacteria grow on meat at the expense of some low molecular weight components. The concentrations of some of these microbial nutrients alter considerably during the development of rigor. Glycolytic intermediates, notably glucose 6- phosphate, are present in low concentration (Fisher and Angustini, 1977). Glucose is not a glycolytic intermediate and the process by which it is formed from glycogen remains speculative (Bendall, 1973). Glucose concentration is a prime determinant of the time of spoilage onset in meat (Gill, 1986).

Surface bacteria do not penetrate into muscle tissue until high bacterial numbers have been attained and overt spoilage has developed. Penetration is delayed because bacterial proteolytic activity is required and proteolytic enzymes are produced only in the late logarithmic phase of growth (Gill and Penney, 1977). Spoilage of meat is, therefore, surface phenomenon unless comminution or

mechanical tenderizing have destroyed the tissue integrity and spread bacteria from the surface throughout the meat surface.

The relative abilities of different organisms to render meat unappetizing to consumers are important for any consideration of spoilage. Visible effects if occur, will be very obvious to a consumer. Most organisms can eventually form visible colonies or slime, but this requires very large numbers to be present. However, if H_2S is formed it will combine with muscle pigments to form sulfmyoglobin, which is seen as green discolouration (Nicol et al.,1970). In addition, H_2S and organic sulphides have highly offensive odour and are organoleptically detectable in very low concentrations. Organisms capable of producing substantial quantities of these substances are therefore, regarded as having a high spoilage potential (Herbert and Shewan, 1976). Other organisms that can produce relatively large quantities of volatile organic compounds such as amines, esters and acetones, are also regarded as being of high spoilage potential (Mc Meekin, 1981).

The development of spoilage varies with the group or species of bacteria dominant in the flora. All the bacteria grow at the expense of the low molecular weight soluble components of meat and the concentration of these substrates and the order in which different groups attack them, can affect the cause of spoilage. Other environmental factors such as the presence or absence of oxygen, can drastically alter the flora composition.

2.8. Control of spoilage in meat

Spoilage control in meat and meat products could be obtained by several methods viz, reduction of pH, addition of glucose, treatment with organic acids, storage in modified atmosphere or vacuum packaging, etc. Most of these methods aim to control spoilage by preservation or by its processing to develop new products. Some of these methods, important from microbial point of view are described below.

2.8.1 Spoilage control by addition of glucose

The glucose content of meat largely determines the critical level of cell density required for spoilage onset. It would seem possible to extend the period of innocuous bacterial growth by increasing the glucose available to the aerobic flora. In case of dark, firm and dry (DFD) meat, this strategy is effective since adding glucose at a level of 100 μg/g to meat previously devoid of glucose, increases the shelf life to that of the normal meat (Newton and Gill, 1978). It is however, doubtful whether any substantial extension of shelf life can be achieved by adding glucose to meat that already has normal content in excess of 100 μg/g. Addition of glucose to meat at concentration of 2% or more by weight did suppress odour and slime formation (Shelef, 1977). This occurred because excess glucose is converted extracellularly by *Pseudomonads* to gluconic and oxogluconic acids (Whiting et al., 1976; Mitchell and Dawes, 1982), and accumulation of these metabolites inhibits growth of the *Pseudomonads*, delaying the appearance of slime (Shelef, 1977; Baura and Shelef, 1980). Off- odours were not formed because amino acid utilization is suppressed by glucose (Jacoby, 1964). Routine treatment of carcasses and cuts with much smaller amounts of glucose could be used to prevent the early spoilage of any undetected DFD muscle during aerobic chiller storage. Addition of glucose to meat that will be vacuum-packaged is positively deleterious as it stimulates the growth of *A.putrifaciens*. Addition of glucose to normal pH meat will only increase the final cell density of the *Lactobacillus* flora and so accelerate the accumulation of volatile acids (Newton and Gill , 1978). Extensive studies have been done on this method of control of spoilage (Barua and Shelef, 1980 ; Gill and Delacy, 1982 ; Jacoby, 1964 ; Mitchell and Dawes, 1982 ; Newton and Gill, 1978 ; Shelef, 1975, 1977 ; Whiting et al., 1976).

2.8.2. Spoilage control by reduction of pH

The Gram-negative psychrotrophs contaminating meat are inhibited by the pH of

normal meat The pH of meat is of little significance in delaying the onset of aerobic spoilage, however, it is of considerable importance for anaerobic spoilage. When the pH is greater than 6.0, growth of *A.putrefaciens* will be accompanied by H_2S production and resultant greening of meat if the number of this organism approaches 10^6 CFU per cm^2. High pH also allows the growth of *B.thermospectra.* This organisms can grow in air at the normal pH of meat but will not grow anaerobically on meat below pH 5.8 (Campbell et al., 1979 ; Grau, 1980). The anaerobic growth of some psychotropic species of *Enterobacteraceae*, including the cold-tolerant pathogen, *Yersinia entercolitica* and common spoilage species, is similarly affected by the pH and lactic acid concentration of normal meat (Grau, 1981). At pH below 4.5 protein, denaturation occurs resulting in brown discolouration of meat. Strains of *Pseudomonas* are notably sensitive to the normal meat pH (Gill and Newton, 1982). Although, many more organisms can initiate growth on meat of high pH, an elevated pH does not seem to result in any significant change in the composition of red meat spoilage flora (Gill and Newton, 1982).

2.8.3. Spoilage control by treatment with organic acid

Treatment of meat with buffered solutions of organic acid is used as a general method for extending the shelf life. Many organic acids have bactericidal or bacteriostatic properties. The lactic acid in meat is itself inhibitory to some groups of organisms (Gill and Newton, 1982 ; Grau, 1980 ; 1981) but the most effective acids are the short-chain length n-fatty acids, such as acetic acid (Geopfert and Hicks, 1969). These acids are biologically active only in the undissociated form and are generally ineffective against meat spoilage organisms above pH 6.0 (Gill and Newton, 1982 ; Grau, 1980,1981).

Lactic acid, acetic acid, citric acids, etc have been extensively used in preserving goat meat, beef and buffalo meats (Saoji et al., 1990; Ravindrernath, 1994;

Webster and Cook, 1984). Also, the ascorbic acid as biological anti-oxidant has been used to stabilize meat colour and reduces the nitrite requirement, and nitrosamine formation (Hood, 1975; Counsell, 1971; Pearson and Tauber, 1984). Verma and Sahoo (2000) reported the extension of shelf life of ground goat meat (chevon) during refrigerated storage by using ascorbic acid. It has been reported that 600 ppm L-ascorbic acid could be the optimum level for pre-blending, which extended the shelf life of ground chevon upto 8 days as compared to 3 days for control under refrigerated conditions. Cheng and Beuchet (1995) also reported the use of organic acids to reduces the growth of experimentally inoculated pathogens viz. *Salmonella spp. Listeria monocytogenes*, *Yersinia enterocolitica* and *E.coli,* etc on the surface of the poultry and animal carcasses. Bajaj et al. (2003) studied the incidence of *Salmonella* in poultry and the meats and growth inhibition of *Salmonella entertidis* by organic acids viz propionic acid, lactic acid, acetic acid, citric acids, etc. The growth inhibitory activity of organic acids against *Salmonella entertidis* has been reported to be in the order as propionic acid < acetic acid < lactic acid < acetic acid < citric acid, etc. The shelf life of beefsteak treated with DL-Lactic acid and antioxidant (0.1% rosemary extract and 0.05% ascorbic acid) has been studied under modified atmosphere (Djenane et al., 2003).

2.8.4 Spoilage control by reduction in temperature

Storage of meat at low temperature retards the growth of all bacteria and thus extends the shelf life. However, the extent to which growth rate decreases with decreasing temperature varies between species and strains of organisms. Psycrotrophic bacteria exhibit growth below 0°C (Larkin and Stokes, 1968). At freezing temperature, the a_w of any medium becomes the same as that of ice. Meat starts to freeze between –1 and –2°C. At temperatures above the freezing point of meat, spoilage by the rapid growth of bacteria usually precludes spoilage by the

slower growing yeasts and molds. Molds spoilage will develops on frozen meat only if it is held for prolonged periods at 2°C or 3°C below its freezing point. If the meat surface reaches higher temperatures, mold spoilage can occur if surface drying inhibits bacterial growth.

Ziauudin et al. (1993) studied the effect of freezing, thawing and frozen storage on microbial profiles of buffalo meat and demonstrated the reduction in microbial counts during frozen storage. For frozen storage of meat, vacuum packaging has been reported to be most effective (Brewer and Wu, 1993).

2.8.5. Spoilage control by drying / dehydration / desiccation

Surface desiccation in meat normally occurs during cooking of carcasses. Water evaporation from the warm carcass significantly reduces the a_w of the surface layers to the extent that checks the bacterial growth. The rate and degree of water loss is greatest at muscle surfaces overlying the thickest area of tissue where cooling is slowest, although the reduction in a_w is more in connective tissue overlying fat. Wide differences between a_w values for surfaces arise because of the complex dynamic situation (Scott and Vickery, 1939 ; Hicks et al., 1955).

With uncontrolled ambient conditions, the surface desiccation associated with cooling usually ensures a lag of about 24 h before any microbial growth commences (Hicks et al., 1955; Nottingham and Wyborn, 1975). Desiccation even delays microbial growth on carcasses held at body temperature for several hours (Wilhelm et al.,1982).

Salting and drying are the oldest methods of meat preservation. Dehydration occurs as a result of moisture loss from meat when it is packed in salt, a process used in dry curing of primal cuts. As the salt penetrates meat, the water activity within the primal meat cut is reduced. The microbiological stability of finished dry cured meats is due to their low water activity, and the presence of nitrate, either added directly or

intrinsic production by certain microorganisms.

Cooked meat may be dried in hot air tunnels at temperatures sufficiently high (above 50°C) to preclude relevant microorganisms. The low temperatures used for freeze-drying of cooked or uncooked meats also preclude microbial growth. Drying of fresh lean meat to 20% moisture inhibits most bacteria, yeasts and molds (Ingram and Simonsen, 1980).

2.8.6. Spoilage control by packaging (modified and vacuum packaging)

The application of vacuum packaging and the importance of conversion of oxygen to carbon dioxide in meat package has been reviewed (Seldman and Durland, 1983). Vacuum packaging reduces the total *psychrophilic* microflora the growth of *Pseudomonas* also reduces, when the oxygen content falls to less than one percent, with concomitant increase in the level of carbon dioxide.

Vacuum packaging of primal cuts of fresh meat is an effective method of reducing storage and trimming losses, labour costs for storage and distribution, besides prolonging the shelf life (Schultsz, 1985). The extract release volume (ERV) of buffalo meat decreases steadily with increase in storage period and beef packed in PVC film showed the highest ERV (Das, 2002). Vacuum packaged meat maintains the lowest thiobarbituric acid (TBA) value on prolonged storage (Arafa and Chen, 1976; Ubersax et al., 1978) and protein breakdown increases as assessed by tyrosine value (TV) during storage (Strange et al., 1977, Kuttinarayanan, 1987).

Vacuum packaging extends the storage life of chilled fresh meat as a result of gradual development of lactic acid population (Rosset, 1982). Lower number of spoilage bacteria on vacuum packed beef has also been reported by Steinhouser et al. (1988) and Bell and Garout (1994). Dushyanthan et al. (2000) investigated the effects of vacuum packaging on the chemical qualities namely ERV, TBA number and TV, and the microbial qualities such as total variable counts (TVC) of beef packed in four

packaging materials and stored at different periods under refrigeration and frozen conditions. Beef packed under vacuum and stored at both chiller and freezer temperatures revealed higher ERV and lower TBA number, TV and TVC. Polyester/polyethylene (PET/PE) and multilayer material have also been reported to be the suitable materials for packaging beef under vacuum and storage at both chiller and freezer temperatures. As the storage period increases under chiller temperature, the ERV, TBA number and TV of beef also increases but the TVC decreases. On the other hand, as storage period increases under freezer temperature the TBA number, TV and TVC of packed beef decreases (Dushyanthan et al., 2000).

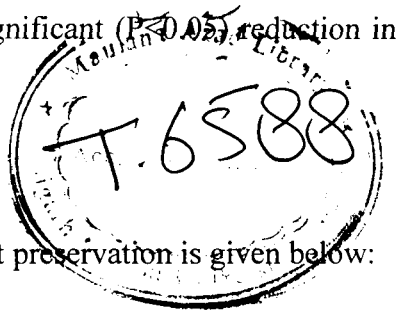
2.8.7. Spoilage control by spray / dip / immersion and pasteurization

Other methods of meat treatment which have been investigated for reducing the initial microbial load on meat include use of water spray (Anderson et al., 1987), hot water dips (Smith and Graham, 1978) and spray (Graham, 1979; Powell and Cain 1987), organic acid dips or sprays (Smulders, 1995) and use of chlorinated water for washing the carcass (Kelly et al., 1981; Cutter and Sirgusa, 1995).

Sachindra et al. (1998) studied the reduction in microbial load on buffalo meat by hot water dip treatment. Treatment of meat cuts with hot water reduces the TPC significantly ($P<0.001$) with a highest reduction of 1.60 log in leg meat and 1.80 log in shoulder meat at 80°C. Also, the dip treatment with hot water reduces the initial bacterial load substantially and improves the microbiological quality of buffalo meat without causing any permanent discoloration.

Whyte et al. (2001) assessed the effect of steam pasteurization and hot water emersion treatment for the microbiological decontamination of broiler carcass. Hot water emersion of broiler thigh pieces for 10 sec at 80°C and 85°C cause significant reductions of 1.09 and 1.25 $\log^{10}$CFU/g in total visible bacteria ($P>0.05$). Statistically insignificant reduction in the counts of total visible bacteria together with levels of

Enterobacteriacea and *thermophilic compylobactor* have been observed upon carcasses expose to atmosphere steam at 90°C for 12 sec. Increase in exposure time of steam pasteurization to 24 sec reportedly results in significant ($P<0.05$) reduction in the counts of *Enterobacteacea* and *Compylobactor.*



2.8.8.Other methods of meat preservation

A brief description of some other methods of meat preservation is given below:

(i) Curing

Curing of meat with salt is a traditional method of meat preservation. Enough scientific literature is available on curing of meat related to curing processes, curing agents, replacement of the meat flora (mainly *Pseudomonads*) by cured meat flora (mainly *Lactobacilli* and *Micrococci*), effects of curing on characteristics of meat, and cured meat products, etc. (Jensen, 1949 ; Callow, 1934 ; Kassai and Karpati, 1963 ; Henrickson et al., 1955; Dyett, 1969 ; Kauffman et al., 1964 ; Draughon et al., 1981; Lucke et al., 1982 ; Woods and Wood, 1982).

Zaemora and Zartizky (1987) reported that addition of sorbate at 350 ppm level reduces microbial contamination remarkably while its dipping extends the shelf life. Gould and Jones (1989) observed that addition of nitrate or nitrite kills microorganisms, prevents germination of surviving spores and improves the colour and flavours. Addition of sodium nitrite or sodium nitrate, alone or in combination with sodium ascorbate or potassium sorbate effectively brings down nitrosamine formation and *C.botulinum* population, and thus reduces the chance of *botulism* (Pearson and Gillett, 1997; Paqutte et al., 1980).

(ii) Use of natural anti-microbial agents in preservation of meat

Different natural substances from various sources have been exploited as preservatives to inhibit microbial growth in food products. These substances have the ability to inactivate undesirable microorganisms and promote the growth of desirable

microorganisms in foods without adversely affecting most of their nutritional and organoleptic properties. The plants, which contain antimicrobial compounds, include spices like cinnamon, clove, coriander, fennel, fenugreek, garlic, ginger, lemon, lime, mustard, onion, pepper, turmeric, etc. Table 2.8 shows the antimicrobial and antioxidant action of some spices used with meat. The natural antimicrobials from plants are phytoalexins, organic acids viz. citric acid, benzoic acid, succinic acid, propionic acid, and lactic acid, medium chain fatty acids, essential oils, pigments and related compounds, hydroxycinnamic acid derivatives, oleuropein and caffein, theophyline and theobromine etc. (Sethi and Sethi, 2002).

Plant extracts intended for use as antioxidants in foods may also have biological effects on bacteria. Dykes (2003) determined the antimicrobial activity of an anti-oxidant ethanolic extract of bearberry leaves (*Arctostaphylos-uva-ursi*), alone and in combination with nisin against 25 food-related bacteria. The combination of nisin and the bearberry extracts reduces the growth of *Brochothrite thermosphacta* and induces sub-lethal injury in *Bacillus cereus* and *Broch thermosphacta.*

(iii) Use of natural antioxidant agents in preservation of meat

Lipids peroxidation is a serious problem in storage of meat and meat products, which leads to loss of shelf life, flavour, colour, texture and safety. Many studies have indicated that lipid oxidation in meat products can effectively be controlled or minimized by the use of antioxidants. These substances can occur as natural constituents of foods, but they can also be added to the products or formed during processing (Table 2.8).

Despite of superior efficacy, low cost and high stability of synthetic antioxidants in food, the suspicion that these compounds may promote carcinogenicity has led to decrease in their use. The use of natural food additives in food industry is preferred by the consumers. From this point of view, carnosine, a

Table 2.8 Antioxidant and antimicrobial action of the some spices used with meat.

Ground spices	Activity	Chief constituents of essential oil	Oleoresin (AI)		Spice equivalents in O/W
			In Lard	Emulsion	
Chilli	---	Capsaicinoids, cartenoids	1.5	2.5	10.9 pwd
Cloves	Antioxidant Antimicrobial	Eugenol	1.8	81.9 -103	13.5
Coriander	---	d-linalool,	1.3	2.5	15.7
Cumin	---	Cumaldehyde, gamma-terpinene	1.3	2.6	13.5 seed
Fennel	Antimicrobial	Anethole	1.3	2.7	14.4 seed
Garlic	Antibacterial	Allium	---	---	10.1 pwd
Ginger	Antioxidant	Zingiberene,cineole, Borneol, geraniol, zingerone	1.8	8.7	15.7
Mustard	Antimicrobial	Allyl-isothiocynate	2.0	2.3	8.6
Turmeric	Antioxidant	Turmerone, curcumin	2-2.9	15.9 -29.6	12.9

AI: Antioxidant Index ---: information not available ; Tsp./oz : Table spoon/ounce; O/W: Oil in water
Adapted from Pearson and Gillett (1997)

natural antioxidant is reported to have tremendous implication in inhibiting lipid oxidation of meat and meat products (Das et al., 2003). It also enhances product quality, safety and shelf life and has several biological actions on human health.

Carnosine (b-alanyl-methylhistidine) is a dipeptide consisting of D-alanine and L-histidine. It is found in the skeletal muscle tissue of most vertebrates and is also found in brain, liver, kidney and other tissues. The concentration of carnosine found in the skeletal muscle (1-25 mM) is capable of inhibiting iron catalyzed lipid oxidation (Decker and Faragi, 1990). Pork, beef, and chicken muscles contain approximately 4, 8 and 15 mM carnosine, respectively. The physiological role of carnosine is unclear but they may act as buffers to prevent large changes in acidity (pH), which otherwise could occur during anaerobic muscle activity that produces lactic acid. It, therefore, maintains the pH of living muscle in physiological range of 6.8-7.4 and also contributes to the buffering capacity of meat post-mortem (Warriss, 2000). It appears to exert a multi-functional effect, acting as a free radical scavenger, a metal chelator and a hydrogen donor (Chen et al., 1993). It is water soluble, thus permitting the inactivation of lipid oxidation catalysts and free radicals in aqueous phase of muscle. Its antioxidant effect is due to contamination of hydrazine of its commercial preparation (Escalante et al., 2002). Carnosine has been shown to be effective antioxidant in refrigerated and frozen salted ground raw and cooked pork (Decker and Crum, 1991), beef homogenate and raw and cooked chicken meat (Neill et al., 1999). It stabilizes the colour of meat and its colour protecting effects are greater than the butylated hydroxytoluene, α-tocophenol or sodium tripoly phosphate (Decker and Crum, 1991, Lee et al. 1999). Carnosine may also indirectly inhibit cholesterol oxidation by removing fatty acid peroxy radicals involved in the initiation of cholesterol oxidation (Neill et al., 1999).

2.9. Hurdle technology

The concept of hurdle technology was evolved by Leistner et al. (1981) after a global review of certain well established traditional food products. Since then various hurdles have been identified, modified and developed to produce several shelf stable products (SSPs). These SSPs do not require refrigeration and can be easily handled to travel long distances.

Hurdle technology exploits scientifically something that has been used for centuries, i.e. the use of more than one factor to preserve foods. For instance, the traditional fruit conservators, use naturally occurring organic acids and sugar as preservative. Cheese manufacture uses lactic acid and salt. Hurdle technology uses a combination of sub-optimal growth conditions, in which each factor on its own is insufficient to prevent the growth of spoilage organisms or pathogens and the combination gives effective control. Fig 2.5 illustrates as to how the hurdle technology can be used to prevent microbial growth. A combination of lower hurdles solves the safety problem with a more acceptable product practical storage temperature.

So far about 50 different hurdles have been identified. These include physical hurdles, physico chemical hurdles, and microbiologically derived hurdles, as listed in Table 2.9 However, it has been observed that these hurdles in combination are better than individual hurdles in prevention of food products. The intensity of individuals hurdles is some times very high to produce undesirable effects on the quality of food products as described in Table 2.10. The concept of hurdle technology, as a combination of several hurdles as techniques has been applied by various researchers for preservation of meat and meat products (Hechelmann et al., 1991, Leistner, 1985 ; Manish and Berwal, 1996 ; Karthikeyan 1997; Wang and Leistner, 1993 ; Modi et al., 1999 ; Himanish and Sumithra, 1998).

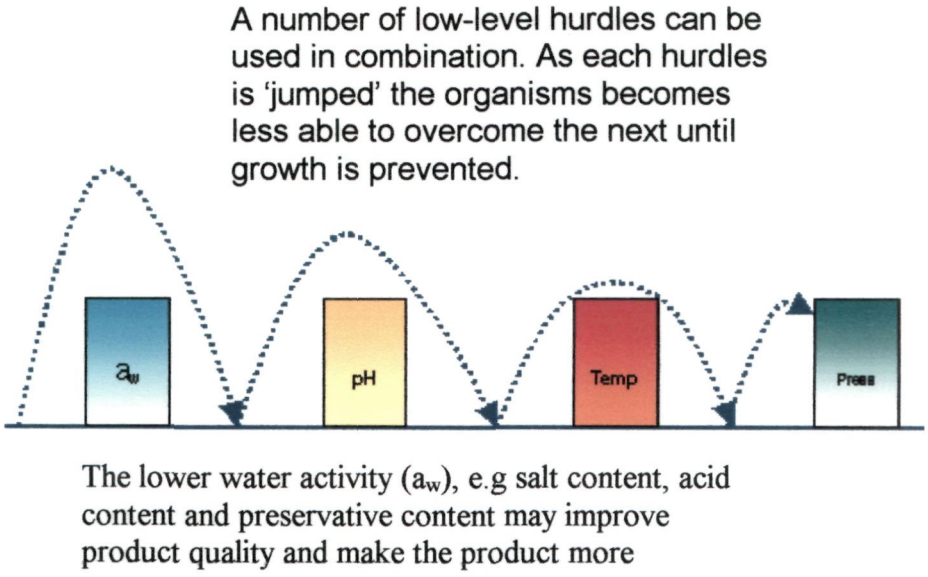


Fig 2.5 How 'hurdles' can be used to prevent microbial growth
Adapted from: Garbutt (1997)

Table 2.9 Types of hurdles used in food preservation

Types of hurdles	Name of hurdles
Physical hurdles	Heat processing (sterilization, pasteurization , blanching)chill temperature, freezer temperature, radiation, ultra-violet radiation, oscillating magnetic field pulses, ionizing radiation, electro-magnetic energy, high electric field pulses, inactivation, microwaves energy radio frequency energy, ultra high-pressure, ultra sonication, packaging photodynamic vacuum packaging, active packaging, edible coating, modified, atmosphere storage, controlled atmosphere storage, hypoboric storage, aseptic, Packaging, micro-structure etc.
Physico-chemical hurdle	Water activity(a_w), pH, redox potential (Eh), nitrate,CO_2, oxygen ,ozone, lactic acid /lactate,acetic acid /acetate, ascorbic acid, sulphite or SO_2, smoking, phosphates, Glucono-d-, lactone (GOL), phenols ,chelators, surface treatment agents, ethanol, propylene, glycol, Millard reaction products(MRPS), spices and herbs, lacto-peroxides, lysozymes etc.
Microbically derived hurdles	Competitive flora, starter cultures, bacteriocins, antibiotics, etc.

Table 2.10 Effects of individual hurdle on quality of products.

Hurdle when used alone	**Undesirable effect on product**
Refrigeration	Chilling injuries/and weight losses,
Freezing	Nutritional /and textural losses, disclouration, enzymatic browning aesthetic changes
Controlled atmosphere storage	Softening, discolouration, retarded spoilage
Pasteurization / Sterilization	Nutritional losses, sensory losses
Drying /dehydration	Discolouration, flavour changes, mold growth etc.
Chemical preservatives	Consumer aversion resistance

Das and Radhakrishna (2001) reported the preservation of mutton curry by hurdle technology, employing various hurdles like water activity (a_w) and oxidation-reduction potential (E_h) to obtain a convenience intermediate moisture product, which after a few minutes of heating in boiling water could be served in curry form. The product has been found to be stable for more than four months at ambient temperature of 27 ± 2°C and more than six months at refrigerated temperature of 3 ± 2°C.

Similarly, Das (2002) has used certain hurdles viz. acidulents (acetic and citric acids), preservatives (ascorbic acid, potassium sorbate and sodium nitrate) in several combinations in the form of six marinade treatments to preserve partially cooked deboned pre-rigor chevon chunks. The effect of the marinade treatments, packaging and storage at 5 ± 2°C on the quality and stability of the meat chunks packaged in pouches of 50 μ polypropylene (PP) film and paper (45 GSM)–aluminum foil (20 μ), polyethylene (37μ), laminate (PFF) have been studied. It is reported that the hurdle processed meat chunks containing moisture ranging from 53.10 to 60.98%, fat 8.91 to 14.76% and pH 4.38 to 6.11 remain stable for a period ranging from 24 to 116 and 28 to 194 days in PP and PFP pouches, respectively. The marinade treatments, packaging and storage period exert, significant ($P<0.01$) effect on shelf life, sensory scores, colour and appearance.

Hurdle technology has also been used for the preservation of minimally processed fruit slices. A combination of mild heat treatment, reduction of water activity, lowering of pH and addition of potassium sorbate and sodium disulphide for producing shelf-stable pine apple slices has been reported by Alzamora et al. (1989). High moisture products from peach, pine apple, mango, papaya, banana, etc. treated with a combination of mild heat treatment, water activity reduction, addition of anti-microbials and packaging in glass flasks or high density polyethylene bags with syrup containing preservatives had a shelf stability ranging from 4 to 8 months (Alzomora et

al., 1993). Vijayanand and Narasimhan (2001) also reported the increased storage life of pine apple, mango and papaya fruit chunks preserved by hurdle technology.

2.10. Hurdle processed meat products

Traditionally several food products with a high moisture content and an acceptable water activity have been developed with addition of humectants viz. salts, sugars, glycerol etc., which reduce water activity with only a small reduction in moisture content. Treatment with a mixer consisting of 35% glycerol, 5% salt, 30% water and 30% beef solids yields meat of a_w 0.75 whereas meats dried without solutes consisting of equal weights of H_2O with a_w greater than 0.95 (Boylan et al., 1976). Some of the intermediate moisture foods developed from meat are presented in Table 2.11.

2.10.1.Dehydrated meat

Drying meat under natural temperature, humidity and circulation of the air, including direct influence of sun rays is the oldest method of meat preservation. Meat drying is a complex process with many important steps, starting from the slaughtering of the animal, carcass trimming, selection of raw material, proper cutting and pre-treatment of the pieces. Reduction in the moisture content of the meat is achieved by evaporation of water from the peripheral zone of the meat to the surrounding air and the continued migration of the water from the deeper meat layers to the peripheral zone (Fig 2.6).

Continuous evaporation and weight looses upto 60 to 70% during drying cause changes in the shape of the meat through shrinkage of the muscle and connective tissue. The meat pieces become smaller, thinner and to some degree wrinkled. The consistency also changes from soft to firm to hard.

In addition to these physical changes, there are also certain specific biochemical reactions with a strong impact on the organoleptic characteristics of the product. Meat

Table 2.11 Analysis of some intermediate moisture foods.

Meat cubes	Water content (%)	Average salt (%)	pH	Water activity
Roasted beef	22.2	3.0	5.75	0.79
Barbecue beef	16.2	2.7	5.05	0.66
Roast pork	22.4	3.6	5.70	0.74
Barbecue chicken	19.7	4.0	5.20	0.70
Chicken -a-la-king	14.9	3.6	5.90	0.61
Beef stew	17.3	3.7	5.80	0.65
Corned beef	16.2	5.4	5.85	0.62
Chili with beans	13.9	2.6	5.65	0.79
Sausage	24.2	4.5	4.90	0.78
Ham	19.9	4.5	5.90	0.72

Adapted from: Karel (1976)

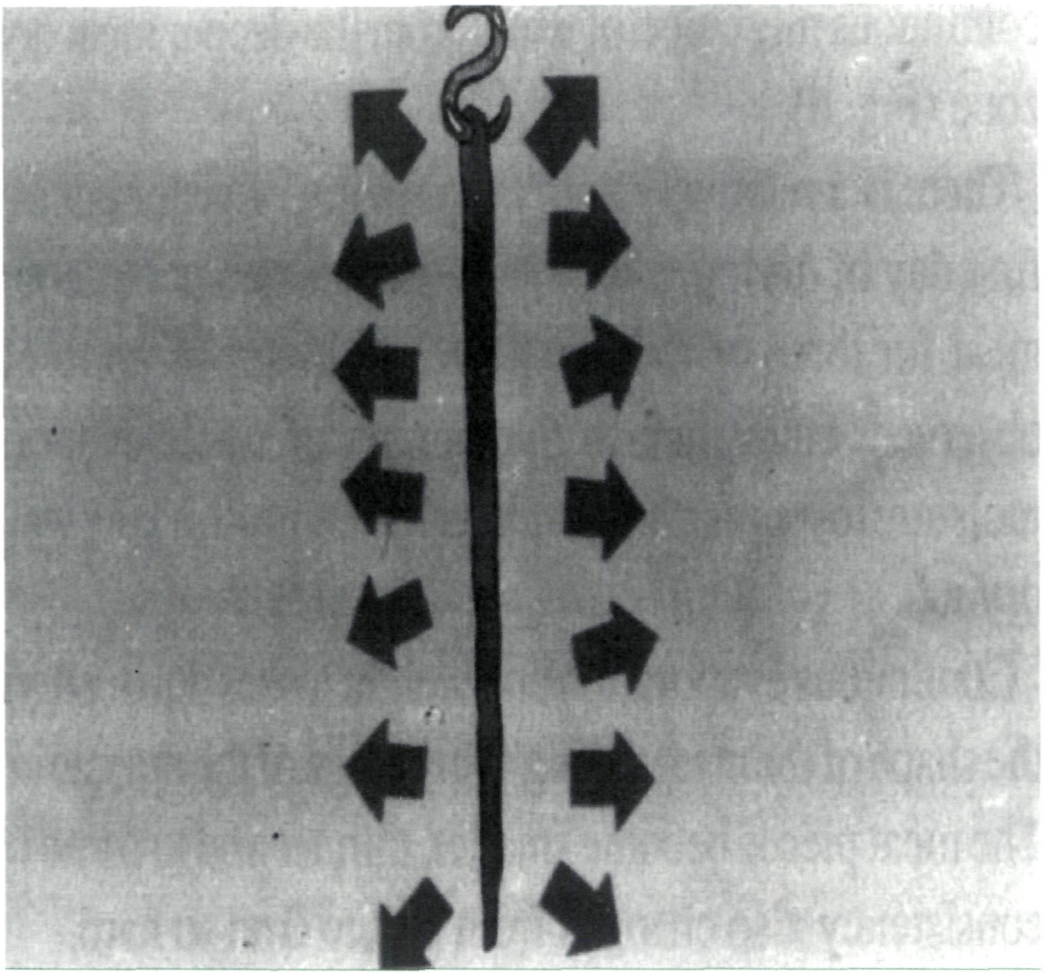


Fig 2.6 Schematic dehydration process of a piece of meat suspended under drying conditions.

used for drying in developing countries is usually derived from unchilled carcasses. Rapid ripening processes occur during the first stage of drying as the meat temperature remains relatively high. The flavour of dried meat is completely different from the characteristic flavor of fresh meat, which is attributed to oxidation of the meat fat.

The dried meats are used as snack food like jerky, beer oticus, tasajo, etc. being commercially produced in U.S.A. Jerky consists of thin strips of salted and cured beef, which can be prepared from primal cuts and sections of coarsely ground beef. Beer sticks are short thin sausages produced from chopped or ground meat, which is mixed with salt, curing agent and spices before being stuffed into edible casing and then heated and dried to achieve shelf stability.

There is very limited published research on dehydrated meat products. The importance of large-scale commercial production of dehydrated meat without compromising its nutritive value and palatability to the fresh commodity has elicited research in the area during 2nd World war (Dunker et al.,1945 and Sharp,1953). Sharp and Rolfe (1958) reported that dehydrated raw meat deteriorates to a greater extent than the cooked product. Meat must be dehydrated immediately after the death of animals or held for some time at temperature below -10° C before dehydration to keep the reactants at the lowest possible concentration.

Various physical factors affect the efficacy of different drying methods used for dehydration of meat. The quality is maintained at a satisfactory level when the drying temperature is 70°C throughout the drying process (Dunker et al., 1945).

The rate of drying is retarded, although to a smaller extent by relatively high fat content. The dehydration time increases if the fat content is about 35% of dry weight. The spongy texture of meat no longer holds the fat, which is dripped away (Sharp, 1953). Dehydrated meat needs to be compressed to exclude pocket of air and

moisture and kept in air tight food containers for longer shelf life. However, because of the Millard reaction dehydrated meat become unpalatable in six months when kept at high temperature (Sharp and Rolfe, 1958). In the presence of O_2, the storage of dehydrated meat at high temperature results in pale and yellow colour. Due to conversion of myoglobin bile pigment, meaty odour develops and fat oxidation occurs giving rise to paint like odour. The fat rancidity does not develop when the moisture content reduces to 1.5 %, however but at such a level, the flavour and texture are likely to be seriously affected. The moisture content of the dehydrated meat is generally too low to permit bacterial growth. However, if it rises above 10%, the mold growth may occur after some time.

2.10.2. Meat powder

There is no record of published literature on meat powder. However, a Sudanese product "Sharamoot" is reported, which is made by dried meat strips, and could be transferred into powdered form for storage (Chang and Pearson, 1992).

2.10.3.Pickles

The process of preserving of food products, specially food and vegetables in common salt and or vinegar is called pickling. Pickling is one of the ancient method of food preservation, which is used as good appetizers and also adds to palatability. Pickles aids in digestion by stimulating flow of gastric juices besides offering flavour, convenience, nutritive value, and variety to the diet. The nutritive value of pickles, however, depends upon the raw food material and method of pickling employed. The information with respect to nutritive value of pickles is not available. However, Lal et al., 1998 reported that food value of cucumber pickle exceed that of egg, rice, fresh onion and fresh tomato.

Different kinds of pickle made from fruits and vegetable are produced in Indian homes in fairly large quantities. They are also manufactured on a large scale

and exported to others countries. Some of the Indian pickles are made from mango, lime, turnip, cabbage, cauliflower and chilies, are popular in several countries. In such pickle mustard/ rapeseed and Sesmum oil, limejuice, vinegar, etc. are used as a pickling medium along with spices and condiments.

Interestingly, the information related to pickles made of lives stock products like meat, egg is scanty. Khanna et al. (2004) studied the shelf stable bone-in-meat pickle from spent hen. Sen and Karim (2003) studied the storage stability of rabbit pickle at room temperature. Shrivastava and Panda (1976) conducted a study on pickling of quail eggs to preserve hard cooked eggs at room temperatures so that they could be transported to far away places without refrigeration. Tipshetty and Panda (1978) later standardized conditions for preparation of quail egg pickles. Yadav and Tanwar (2003) further studied the microbial profile of quail eggs pickled in different pickling media viz. mustard oil, 3% acetic acid, 3% propionic acid and 4% tartaric acid. The microbial profile of pickled quail egg evaluated at different intervals of storage periods revealed inorganic acid media cause decrease of microbial count as compared to that on 0^{th} day of storage, except in case of propionic acid medium where the microbial count first decreased up to the 38^{th} day and than increased during remaining days of storage period. In case of oil based pickling medium, the microbial count of eggs pickle reportedly increases during storage period of 90 days.

As far as meat is concerned, a limited information is available on meat pickles. However, pickling is a known method of meat curing. Typical meat curing pickle used in commercial ham preparation consists of salt, sugar, sodium nitrite and phosphates. This method of pickle curing is also used to produce corned beef in large extent. The usual meat pickles are 6% to 7% brine and the products has a mild flavour. However, pickles need to be stored in airtight containers (Pearson and Gillette, 1997).

Materials & Methods

List of chemicals

Name of chemicals	Source
Acetic acid	Qualigens, India
Agar –agar	Hi-Media, India
Ammonium phosphate	Hi-Media, India
Baird-parker medium	Hi-Media, India
Beef extract	Hi-Media, India
Boric acid	Qualigens, India
Brilliant green	SRL, India
Streptomycin	Cipla, India
Egg yolk tellurite enrichment	Hi-Media, India
Gelatin agar	Hi-Media, India
Hydrochloride acid	Qualigens, India
MacConkey Agar	Hi-Media, India
Nutrient agar	Hi-Media, India
Peptone	Hi Media, India
Sodium chloride	Hi-Media, India
Sodium hydroxide	Hi-Media, India
Trichloroacetic acid	Hi-Media, India
Thiobarbituric acid	Qualigens, India
Yeast extract	Hi-Media, India

Media composition and buffer

Media	Quantity (gl^{-1})
Baird-parker medium	
Tryptone	10.0
Beef extract	5.00
Yeast extract	1.00
Sodium pyruvate	10.0
Glycine	12.0
Lithium chloride	5.00
*Egg yolk tellurite enrichment	50.0 ml
Agar	20.0
pH	7.0 ± 0.2
*Add egg yolk tellurite enrichment in sterile molten medium	
MacConkey's agar	
Peptone	20.0
Lactose	10.0
Bile salt No 3 or bile salt mixture	1.50
Sodium chloride	5.00
Neutral red	0.03
Crystal violet	0.001
Agar	13.50
pH	7.1 ± 0.2

Milk agar

Skim milk powder	100.0
Peptone	5.0
Agar	15.0

Nutrient agar

Beef extract	3.0
Peptone	5.0
Sodium chloride	5.0
Agar	15.0
pH	6.8 ± 0.2

Potato dextrose agar

Potato infusion	200.0
Dextrose	20.0
Agar	20.0
pH	5.6 ± 0.2

For yeast and mold, streptomycin (40µg/ml) was added

Tributyrin agar

Peptone	5.0
Yeast extract	3.0
Tributyrin	10.0 ml
Agar	15.0
pH	5.6 ± 0.2

Buffer	**Quantity (gl^{-1})**
Phosphate–buffered saline (PBS)	
Sodium chloride	7.650
Disodium hydrogen phosphate, anhydrous	0.724
Potassium dihydrogen phosphate	0.210
pH	7.4 ± 0.2

3.1 Collection of raw meat

Deboned meat from the thigh and shoulder portions of a freshly slaughtered adult (10 years of age), Murrah buffalo carcasses were collected. The samples were immediately packed in low-density polyethylene bags and transferred to the laboratory in ice for further processing. The samples were stored in a deep freezer at -20°C until used.

3.2 Product development

Improved quality hurdle processed, two buffalo meat products were prepared under different treatments conditions, as described below:

3.2.1 Hurdle processed meat pickles

3.2.1.1 Preparation of stock of spices / condiments

Initially, the stocks of all spices / condiments used for pickle preparation were prepared. Briefly, 100 g each of ungrounded spices viz. clove, cinnamon, turmeric, garlic, red chilly, fennel, coriander, fenugreek, cumin, nigella (Table 3.1) and salt (iodine free) were separately mixed with 500 ml of 3% acetic acid for 10 min and dried at 80°C for 8h. Each of the dried spices was than grounded to a fine powder under aseptic condition. The stocks of spices/condiments were stored in sterile, properly labeled glass bottles at ambient temperature.

3.2.1.2. Preparation of pickles

Fat free raw meat pieces (1kg) were washed thoroughly under running tap water, and left in hot water for 30 min. The cleaned meat pieces approximately (1cm x 1cm) were deep fried in selected oil medium. For preparing different types of pickles, the fried meat pieces were mixed with specific combinations of spice/ condiments mixture and submerged in specific medium. The common ingredients of pickles were 25g/kg each of coriander, cumin, fenugreek, red chilly and turmeric and 10g/kg of nigella and tartaric. Fennel and table salt were added at concentration of

Table 3.1. Common, trade and botanical name of the ingredients used in pickle preparations.

Trade name	**Local trade name**	**Botanical name**
Cardamom	Badi-elaychi	*Elettaria cardamomum L.Maton*
Cinnamon	Dalchini	*Cinnamomum zeylanicum Nees*
Clove	Laung	*Syzygium.araomaticum*
Coriander	Dhania	*Coriandrum sativum L*
Cumin	Zira	*Cuminum cyminum L*
Fennel	Saunf	*Foeniculum vulgare*
Fenugreek	Methidana	*Trigonella foenum*
Garlic	Lassan	*Allium sativum*
Ginger	Adrak (fresh)	*Zingiber officinale*
Mustard	Sarson	*Brassica hirta*
Nigella	Kalaongi	*Nigella sativum L*
Red chilly	Lal-mirch	*Capsicum frutescens*
Turmeric	Haldi	*Curcuma longa*

Table 3.2 Classification and composition of the meat pickles developed.

Types of Meat pickle	Treatments	Ingredients	Pickling medium
I	**Control** (Untreated pickle)	Coriander, Cumin, Fennel, Fenugreek, Nigella, Red chilly, Tartaric and Turmeric	Soybean oil
	Natural products		
II	Cinnamon	Coriander, Cumin, Fennel, Fenugreek, Nigella, Red chilly, Tartaric and Turmeric	Soybean oil
III	Clove	Coriander, Cumin, Fennel, Fenugreek, Nigella, Red chilly, Tartaric and Turmeric	Soybean oil
IV	Garlic	Coriander, Cumin, Fennel, Fenugreek, Nigella, Red chilly, Tartaric and Turmeric	Soybean oil
V	Mustard	Coriander, Cumin, Fennel, Fenugreek, Nigella, Red chilly, Tartaric and Turmeric	Mustard oil
VI	Turmeric	Coriander, Cumin, Fennel, Fenugreek, Nigella, Red chilly, Tartaric and Turmeric	Soybean oil
	Synthetic preservatives		
VII	Sodium nitrite	Coriander, Cumin, Fennel, Fenugreek, Nigella, Red chilly, Tartaric and Turmeric	Soybean oil
VIII	Potassium sorbate	Coriander, Cumin, Fennel, Fenugreek, Nigella, Red chilly, Tartaric and Turmeric	Soybean oil
IX	Acetic acid	Ginger, Garlic and Red chilly	Acetic acid

50g/kg. Ginger and garlic (20g/kg) were supplemented only in case of acetic acid treated pickle. This base composition of pickle was taken as a control (Type-I) pickle. Treatments with 2% of spices and condiments were given to develop eight different types of pickles as specified in Table 3.2. Soybean oil was used as a medium for meat pickles (Types I-VIII) except Type-V pickle in which mustard oil was used. Moreover, acetic acid was used as medium in Type-IX pickle. Each type of pickles was packed in HDPE and glass jars and stored at ambient temperature for studying their shelf life and quality attributes as a function of time.

Out of nine specific meat pickles, the pickles (Types II-VIII) were supplemented with five different natural products viz. cinnamon (2%), clove (2%), garlic (2%), mustard (2%), and turmeric (2%). However, the Types VII and VIII meat pickles were supplemented with synthetic preservatives viz. sodium nitrite (0.02%) and potassium sorbate (0.025%). The concentrations of treatments (spices/ condiments) were determined based on the minimum inhibitory concentration (MIC) of these preservatives with pure cultures of common bacterial contaminants of meats.

Type IX pickle (acetic acid treated meat pickle) was processed in an entirely different manner. In brief, 1kg of thoroughly washed, fat free raw meat pieces (1cm) were dipped in hot water for 30 min. The meat was then boiled with mixture cardamom, cinnamon and cloves (0.2%) for 30 min. The boiled meat was marinated for 1 h with the semi-liquid mixture of garlic, ginger, red chilly and table salt (non-iodized). The meat pieces were then fried in (soybean) oil and dipped in pre-sterilized 1000 ml brine solution (6% acetic acid).

3.2.2 Hurdle processed dehydrated meat powder

The dehydrated buffalo meat powder was developed using combinations of natural/synthetic preservatives. The effect of processing, packaging and storage period on the quality and stability of meat powder has been investigated. Minced meat

(1kg) was washed twice with tap water, and then dipped in hot water for 30 min. It was then dried in hot air oven initially at 180^0C. The sample was checked periodically and rotated for uniform drying. With the change in moisture content of the meat, the temperature was gradually reduced from 180^0C to 60^0C. The completely dehydrated meat was then grinded to obtain fine powder. The meat powder was then separately treated with natural and synthetic preservatives viz. clove (2%), turmeric (2%), and potassium sorbate (0.025%). The treated meat powder was packaged in combination film and autoclaveable polythene.

3.3 Sample packaging

Aliquots of 200g of each type of meat pickle were packed in HDPE and glass jars. Similarly, the treated and control meat powder of (250g) were packed in combination film and autoclaveable polythene, following the three methods:

(i) Atmosphere packaging followed by heat-sealing using a paddle operator machine.

(ii) Vacuum packaging.

(iii) Modified atmosphere packaging (MAP) by CO_2 and N_2 flushing methods.

3.4 Evaluation of organoleptic qualities

The colour, odour, juiciness, taste, palatability and texture characteristics of the fresh and packed meat pickles as well as meat powder and kabab were evaluated organoleptically based on nine point hedonic scale; where in 9 points were given to 'like-extremely' characteristics and 1 to 'dislike extremely'(Table 3.3). The organoleptic evaluation of meat samples was done at an interval of 20 day during 120 days storage period. The test performa was developed and provided to atleast nine individuals at the time of evaluation for obtaining statistically significant data.

3.5 Microbiological Analysis

Samples were prepared according to the procedure of Andrews et al. (1998). Briefly, 10 g sample was homogenized in 90ml of butterfield phosphate buffer (BPB)

Table 3.3 Nine point hedonic scale for organoleptic evaluations

Sensory attributes	**Scores**
Like extremely	9
Like very much	8
Like moderately	7
Like slightly	6
Neither like nor dislike	5
Dislike slightly	4
Dislike moderately	3
Dislike very much	2
Dislike extremely	1

Adapted from: Ranganna (1994)

and serial diluted upto 10^{-6} dilution. From each dilution, 0.1ml of sample was spread onto selective media plates under aseptic conditions. Total plate counts, as well as coliform, yeast and mould, proteolytic, lipolytic counts were determined on nutrient agar, Mackonkey agar, PDA, milk agar, and tributyrin agar respectively. The plates were incubated at 37°C for 24 to 48 h. The bacterial and fungal counts were determined and presented as described by APHA (1995).

3.5.1 Determination of minimum inhibitory concentration (MIC) of preservatives

The antimicrobial activities of the extracts of cinnamon, clove, garlic, turmeric, mustard were assessed by agar diffusion method as described by Arora and Kaur (1999). The spices extracts were prepared according to method of Shashikanth et al. (1981). All the spices were grounded with twice the weight of sterilized distilled water in warring blender. The homogenate was allowed to stand for 10 min and filtered through sterile cheese cloth. The filtrate was transferred to clean sterilized tubes and stored at 4°C till used. For MIC determination, 0.2ml of each filtrate in the range of 0.5 to 5% was tested against the pure cultures of two Gram-positive (*Staphylococcus aureus*, *Listeria monocytogenes*) and two Gram-negative species (*Escherichia coli* and *Salmonella enteritidis)* bacteria, commonly associated with meat spoilage, for growth inhibition.

In brief, wells (8mm) in diameter were cut in nutrient agar plates using sterile stainless steel borer and filled with 0.2ml of various spice extracts. The plates inoculated with specific test microorganisms, were then incubated at 37°C for 24 h. The diameters of any resultant zones of inhibition appeared at various concentrations were measured. The bacteria showing a clear zone of >10 mm were considered to be positively inhibited.

3.6 Physico-chemical analysis

3.6.1 pH measurement

For pH measurement of meat sample, 10g of minced meat was homogenized in 50ml distilled water in waring blender (Yorco, India). The homogenate was transferred to a clean beaker and the pH of the suspension was measured using digital pH meter (Thermo Orion, USA)

3.6.2. Thiobarbituric acid (TBA) number

TBA number was measured according to the method of Strange et al. (1977). TCA extracts of meat and meat products (5ml) were mixed with 5ml of TBA reagent (0.29% in 90% acetic acid) prepared as described by Pearson (1973). The reaction mixture was kept in a water bath at 100°C for 30 min, along with a reagent blank containing 5ml of TCA and 5ml of TBA reagent. The absorbance of the colour developed was measured at 530 nm using visible spectrophotometer (Thermo spectronic, USA) and reported as TBA number.

3.6.3. Moisture content

Moisture content was determined following the standard method (AOAC, 1995). In brief, 10g of meat sample was weighed in a tared dish. The dish with its content placed in hot air oven at 150 $\pm$ 5 °C for 16 h. It was then transferred to a desiccators for cooling. The dish was weighed again and the moisture content of meat/meat products determined using the formula as:

$$\textit{Moisture}\ \text{content}\ \% = \frac{\text{Loss in weight of sample}}{\text{Initial weight of sample}} \times 100$$

3.6.4. Ash content

Ash content of the sample was determined following the standard method (AOAC, 1995). In brief, 2g meat/meat product samples in a crucible were kept for 6 h in a furnace maintained at 850°C. The hot crucible was carefully transferred to a

desiccators until cooled and finally weighed. The estimation of ash content of meat samples/meat products was done, using the formula given below:

$$\text{Ash content \%} = \frac{\text{Final weight of ash}}{\text{Initial weight of sample}} \times 100$$

3.6.5 Protein content

Protein content was determined based on the amount of total nitrogen in the sample following the method of Ranganna (1994) using the relationship: Amount of protein in the sample = total nitrogen x 6.25.

In brief, 5g of finely minced sample was taken into a digestion flask. To this, 2g catalyst mixture and 10 ml concentrated sulphuric acid were added. The mixture was heated until frothing ceased and become colourless. The digested liquid was filtered and the volume was adjusted to 250 ml. The digested sample (10 ml) was then mixed with 40 ml of 30% NaOH in the tube and steam passed through the distillation tube. The ammonia liberated from the reaction mixture was absorbed in 10ml of 20% boric acid solution. Distillation was continued for five minutes. The solution was then titrated against N/100 HCl using mixed indicator. A parallel blanks also run and titrated as described. Percent nitrogen was calculated as:

$$\text{N (\%)} = \frac{\text{(Sample} - \text{blank) x N of HCl x 14 x Vol made up of the digest}}{\text{Aliquot of digest x weight of sample x1000}} \times 100$$

3.6.6 Fat estimation

Fat estimation was performed according to method described in AOAC (1995). 20g of meat/meat product sample on a porous paper in a thimble and transferred to extraction tube in Soxhlet apparatus. The extraction was performed for 20 h and the extract collected. The volatile solvent was evaporated and the dried residue matter was weighed. Fat content of sample was determined as:

$$\text{Fat content (\%)} = \frac{\text{Weight of residue}}{\text{Weight of sample}} \times 100$$

3.7 Textural analysis

Textural analysis of meat pickles, and kababs prepared from buffalo meat powder was done using TAHD type texture analyzer (SMS, England). In case of meat and meat products, 'Compression plate' was used as a probe for measurement of hardness/tenderness of meat pickles and kabab prepared from buffalo meat powder.

The analysis was performed at the texture profile analysis (TPA) setting specified as in Table 3.4. The pre-test speed (2.00 mm/s) ; test speed (2.00 mm/s) ; post-test speed (5.00 mm/s). The test conducted on (50%) strain with an auto trigger force of (5g) . The data computed using 'texture expert software.

3.8 Particle size determination

The average particle was measured according to the methods of Farooqui (1988) and McCabe et al. (2001). In brief, to measure the average particle size the dry sieve analysis was performed. The sieves were fitted with standard size screens ranging from 101.4 to 0.038 mm. In the present study, the screens selected are in accordance with British Standard Institution (BS–410). The average particle size for a mixture of particles was determined using the formula as given below:

$$D_s = \frac{1}{\Sigma \frac{xi}{Dpi}}$$

Where,

xi = Mass fraction retained
Dpi = Average particles diameter in crements
$\mathbf{D_s}$ = Volume surface mean diameter

Table 3.5 Texture profile analysis (TPA) setting

Pre-test speed	2.00 mm/s
Test speed	2.00 mm/s
Post-test speed	5.00 mm/s
% Strains	50.0 %
Count	2
Trigger	5 g
Load cell	50 g
Probes	P/100- 100mm (Compression plate)

3.9 Data analysis

The data of physicochemical, microbiological and textural characteristics of pickles and meat powder were statistically analyzed by two way ANOVA (Mandal and Nambiar, 2002).The mean values of three replicates with standard deviation are presented.

Results & Discussion

This chapter presents the data related to development and preservation of two hurdle processed buffalo meat products viz. meat pickles and meat powder. The following sub-sections describe the physico-chemical, microbiological, organoleptic and textural characteristics of above two products. The changes in characteristics of the products during preservation in different packaging materials have also been discussed with a view to assess the shelf life of these products.

Product one: Hurdle processed meat pickles

4.1. Development and quality evaluation of buffalo meat pickles

In this study, nine different types of meat pickles were developed using different hurdle parameters including, preservatives (chemical and natural spice/condiments) and packaging materials (HDPE and glass jars). Figs. 4.1 and 4.2 depicts the physical appearance of the oil based and acetic acid treated meat pickles, respectively. The natural products such as cinnamon, clove, garlic, turmeric and synthetic preservatives like potassium sorbate, sodium nitrite and acetic acid have been used as treatments. The meat pickles with these treatments were developed in soybean oil. Besides, the mustard and acetic acid treated meat pickles were developed in the same mediums, respectively. The process of preparation of these pickles has already been discussed in chapter 3. The prepared pickles were packed in HDPE and glass jars and stored at ambient temperatures during March to August with in the temperature range of 30°C to 35°C.

The physico-chemical, microbial, organoleptic and textural characteristics of meat pickles were evaluated both in fresh and preserved conditions and the results are presented below:

4.1.1 . Physico-chemical characteristics

The physico-chemical parameters such as, pH, protein, fat, ash content and TBA numbers, were examined and their impact on quality of meat pickle assessed.

Fig 4.1 Oil based meat pickles packed in HDPE and glass jars.

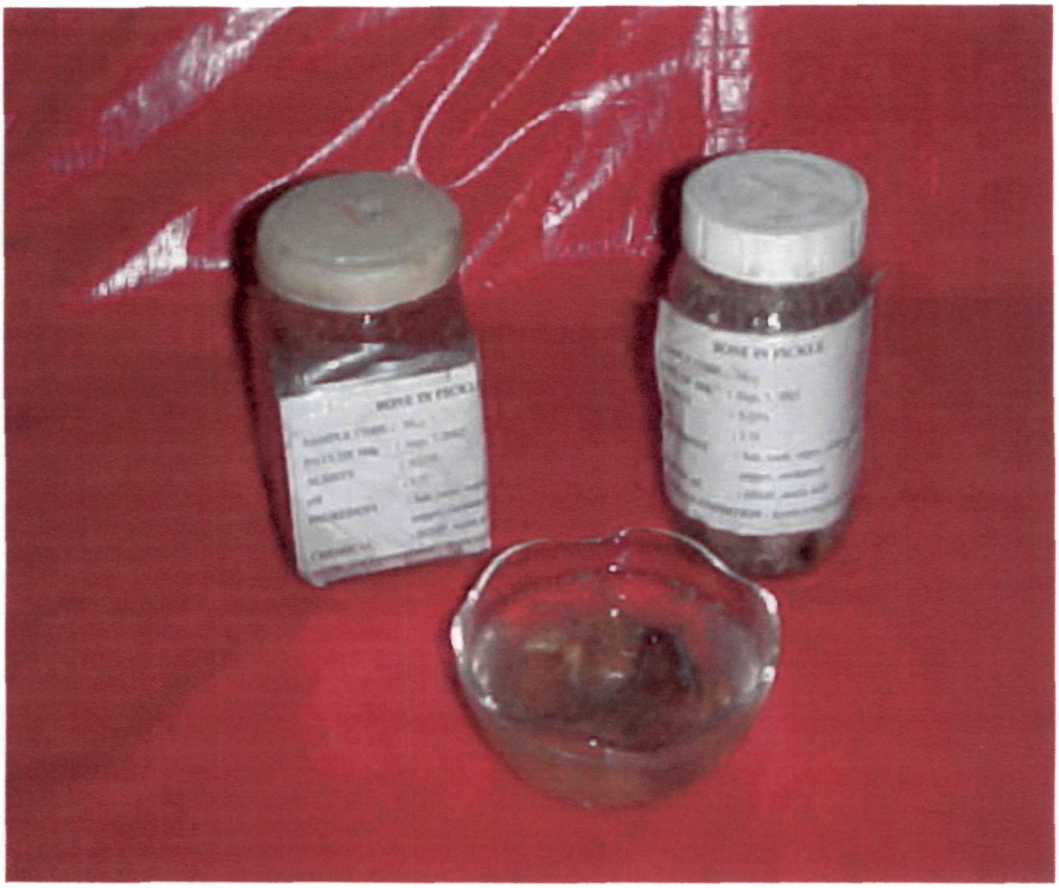


Fig 4.2 Acetic acid treated meat pickle packed in HDPE and glass jars

4.1.1.1 pH

pH is the most commonly measured quality attribute in meat and meat products, which affects the keeping quality and sensory traits. An earlier study has demonstrated high correlation between the pH and the rate of spoilage (Blixt and Borch, 2002). The relationship between pH and level of glycogen is not linear possibly due to inactivation and disappearance of certain cofactors and enzymes at lower pH values (Sahlin,1978). The rate of pH decline depends upon ATP metabolisms (Bendall,1973). Beef is considered to have a normal pH value in the range of 5.4 to 5.8. However, the post-mortem breakdown of glycogen results in reduction in the pH of the meat. Since sensory and technological meat qualities depend on the rate and amplitude of the pH decline. Thus, the variations in pH as a function of time have been measured in buffalo meat pickles during 120 days storage at room temperature.

The data shown in Table 4.1 indicate the time dependent reduction in the pH of the packaged control (untreated) meat pickles from pH 4.9 to 3.37 and 4.13 at the end of 120 days storage in HDPE and glass jars, respectively. The low pH of meat in pickle on day 1 corroborates with the observation of Lemay et al.(2002) that the pH of meat slurry under go reduction from pH 4.8 to 4.37 after 14 days of storage. It has been suggested that the pH of the meat slurry is lower than the meat surface. Nevertheless, the treatments with both the natural and synthetic preservatives caused significant reduction in the pH values of meat pickles (Table 4.1). The reduction in pH was noticed invariably with all the eight treatments in a time dependent manner upto 120 days storage period at ambient temperature. The decrease in meat pH may be due to frying of meat. Sen and Karim (2003) have also reported that the fried rabbit meat pickle has a 4.88 as compared to boiled form of meat pickle (pH 5.0). The data shown in Table 4.1 also demonstrated that the changes in meat pH initiated after 20

Table 4.1 Effect of treatments on pH of buffalo meat pickles during storage of 120 days at ambient temperature.

Samples		Change in pH as a function of storage time (days)						
		0	20	40	60	80	100	120
Control (untreated)	P_1	4.90 ± 0.61	4.50 ± 0.30	4.37 ± 0.23	4.13 ± 0.06	4.37 ± 0.29	3.83 ± 0.46	3.37 ± 0.12
	P_2	4.90 ± 0.61	4.53 ± 0.15	4.37 ± 0.31	4.30 ± 0.27	4.40 ± 0.17	4.03 ± 0.50	4.13 ± 0.06
Natural products								
Cinnamon	P_1	4.53 ± 0.49	3.30 ± 0.17	3.33 ± 0.06	3.30 ± 0.06	3.30 ± 0.17	3.33 ± 0.25	3.33 ± 0.25
	P_2	4.53 ± 0.49	3.30 ± 0.17	3.30 ± 0.12	3.33 ± 0.25	3.30 ± 0.06	3.33 ± 0.06	3.37 ± 0.31
Clove	P_1	4.57 ± 0.49	3.57 ± 0.12	3.50 ± 0.06	3.53 ± 0.10	3.73 ± 0.15	3.37 ± 0.23	3.20 ± 0.06
	P_2	4.57 ± 0.49	3.57 ± 0.12	3.57 ± 0.12	3.57 ± 0.12	3.57 ± 0.12	3.57 ± 0.12	3.57 ± 0.12
Garlic	P_1	4.33 ± 0.21	3.83 ± 0.75	3.60 ± 0.17	3.60 ± 0.17	3.57 ± 0.40	3.50 ± 0.36	3.50 ± 0.10
	P_2	4.33 ± 0.21	3.87 ± 0.81	3.87 ± 0.15	3.73 ± 0.12	3.73 ± 0.35	3.73 ± 0.12	3.73 ± 0.25
Mustard	P_1	4.53 ± 0.49	4.53 ± 0.29	4.50 ± 0.20	4.33 ± 0.12	4.23 ± 0.15	4.20 ± 0.10	4.13 ± 0.25
	P_2	4.53 ± 0.49	4.30 ± 0.20	4.27 ± 0.06	4.27 ± 0.06	4.27 ± 0.15	4.27 ± 0.06	4.17 ± 0.06
Turmeric	P_1	4.20 ± 0.17	4.23 ± 0.74	4.20 ± 0.14	4.23 ± 0.06	4.27 ± 0.15	4.03 ± 0.21	3.97 ± 0.15
	P_2	4.20 ± 0.17	4.20 ± 0.10	4.27 ± 0.06	4.27 ± 0.06	4.27 ± 0.15	4.07 ± 0.15	4.00 ± 0.17
Synthetic preservatives								
Acetic acid	P_1	3.50 ± 0.20	3.40 ± 0.27	3.33 ± 0.15	3.20 ± 0.70	3.20 ± 0.15	3.17 ± 0.06	3.17 ± 0.16
	P_2	3.50 ± 0.20	3.40 ± 0.27	3.33 ± 0.15	3.23 ± 0.74	3.23 ± 0.15	3.20 ± 0.06	3.20 ± 0.17
Potassium sorbate	P_1	4.87 ± 0.32	4.77 ± 0.21	4.70 ± 0.17	4.70 ± 0.21	4.70 ± 0.26	3.97 ± 0.38	3.50 ± 0.61
	P_2	4.87 ± 0.32	4.77 ± 0.15	4.70 ± 0.17	4.70 ± 0.17	4.67 ± 0.23	4.03 ± 0.49	3.83 ± 0.58
Sodium nitrite	P_1	4.67 ± 0.47	4.63 ± 0.23	4.57 ± 0.40	4.40 ± 0.30	4.40 ± 0.21	3.80 ± 0.56	3.73 ± 0.45
	P_2	4.67 ± 0.47	4.73 ± 0.21	4.73 ± 0.12	4.73 ± 0.12	4.57 ± 0.31	4.20 ± 0.36	4.17 ± 0.35

The data represent the mean ± SD of three replicates; ANOVA Table A.1 is given in annexure; P_1 =HDPE jar, P_2 =Glass jar
Treatment CD at 5% = P_1 (0.01) ; P_2 (0.01) ; Storage CD at 5% =P_1 (0.01) ; P_2 (0.01)

days of storage occur relatively slower in glass jar vis-à-vis HDPE jars . Overall the pH profile of meat pickles showed the pH changes with different treatments in the order as potassium sorbate > sodium nitrite > clove > cinnamon > mustard > garlic > turmeric > acetic acid. The data revealed that the natural products supplemented to the pickles resulted in slightly higher reduction in pH values as compared to synthetic preservatives, except acetic acid. The acetic acid treated meat pickle has the lowest pH of 3.5, which reduced further during storage at room temperature and attained the values of pH 3.17 and pH 3.2 in HDPE and glass jars, respectively. The action of acetic acid as an acidulates results in reducing the transmembrane gradient, which influences the proton motive force and hydrogen and hydroxyl ion concentrations both external and internal to cytoplasmic membrane. More hydrogen ions outside the membrane results in lowering of pH, which serves as one of the hurdles for microbial growth.

Nevertheless, the low pH of meat pickles is an important factor for checking the bacterial growth besides maintaining some of the physical attributes of the meat. Meat binds least water at pH value around 5.0 to 5.1, which is the pH value at which the electrical net charge of myofibrils and myofibrillar volume is minimal. Therefore, at lower pH values water is driven towards the extracelluar spores, and consequently meat shows lower water holding capacity . Unlike low pH the higher ultimate pH is associated with increased juiciness and decreased flavour (Dransfield and Jones, 1981). In general, the meat with the higher ultimate pH, are darker and more susceptible to spoilage. The meat pickles specified in Table 4.1, in spite of having lower pH exhibited increased tenderness with storage. Storage is associated with an increase in tenderness and flavour of the meat due to the degradation of protein and lipids (Quali and Talmant, 1990 and Dransfield, 1994).

4.1.1.2.Protein Content

The raw buffalo meat has an average protein content of 19.32 % at a pH of 5.89. In contrast, the protein contents of meat pickles, have been determined to be in the range between the minimum value of 20.70% for control samples and maximum value of 21.20% for clove treated samples (Table 4.2). The data indicate a significant ($P<0.05$) improvement in protein content due to pickling. This increase in protein content of pickles is mainly attributed to protein values of pickling medium and ingredients. The protein content of soybean is higher than mustard. Therefore, the protein contents of all samples, including the control and spice treated pickles, developed in soybean oil medium, were higher than the raw meat. The variations in protein contents of control and spice treated pickles may again be attributed to protein contents of individual spices used in pickling medium. For instance, the cloves has been reported to have 20% protein. Comparative analysis of pickle meat protein content revealed that the protein value varies in the decreasing order as clove > turmeric > cinnamon > garlic > potassium sorbate and sodium nitrite (individually) > acetic acid > mustard.

Protein contents of meat pickles decreased to varying extent during 120 days storage at ambient temperature. It appears that the spices in pickles help maintain the protein content value of the meat in pickle as compared to control, which has shown significant reduction in protein content after 120 days of storage. This is due to protein degradation upon ageing of meat as a natural process during storage. Significant ($P<0.05$) reduction of protein content in acetic acid was noticed both in HDPE jar (6.86% loss) and glass jar (6.63% loss) packed samples. In all other treatments, the decrease in protein contents of pickles during storage was insignificant ($P>0.05$).

Table 4.2 Effect of treatments on protein values of buffalo meat pickles during storage of 120 days at ambient temperature.

Samples		Protein (%) as a function of storage time (days)						
		0	20	40	60	80	100	120
Control (untreated)	P_1	20.70 ± 1.06	20.70 ± 1.08	20.67 ± 1.12	20.60 ± 0.20	20.27 ± 0.12	20.03 ± 0.21	19.77 ± 0.06
	P_2	20.70 ± 1.06	20.70 ± 1.11	20.67 ± 1.06	20.63 ± 0.15	20.33 ± 0.12	20.17 ± 0.35	19.84 ± 0.05
Natural products								
Cinnamon	P_1	21.07 ± 0.31	21.07 ± 0.06	21.07 ± 0.12	21.07 ± 0.06	21.07 ± 0.31	21.00 ± 0.00	21.00 ± 0.00
	P_2	21.07 ± 0.31	21.07 ± 0.12	21.07 ± 0.31	21.07 ± 0.06	21.07 ± 0.12	21.07 ± 0.06	21.07 ± 0.12
Clove	P_1	21.20 ± 0.17	21.20 ± 0.10	21.20 ± 0.17	21.17 ± 0.06	21.17 ± 0.06	21.17 ± 0.12	21.13 ± 0.15
	P_2	21.20 ± 0.17	21.20 ± 0.10	21.20 ± 0.17	21.20 ± 0.17	21.20 ± 0.10	21.20 ± 0.17	21.20 ± 0.17
Garlic	P_1	21.00 ± 0.10	21.00 ± 0.27	21.00 ± 0.30	21.00 ± 0.27	21.00 ± 0.17	20.97 ± 0.23	20.93 ± 0.21
	P_2	21.00 ± 0.10	21.00 ± 0.17	21.00 ± 0.30	21.00 ± 0.27	21.00 ± 0.17	21.00 ± 0.17	21.00 ± 0.30
Mustard	P_1	20.83 ± 0.51	20.80 ± 0.46	20.63 ± 0.21	20.60 ± 0.17	20.57 ± 0.15	20.47 ± 0.21	20.40 ± 0.30
	P_2	20.83 ± 0.51	20.83 ± 0.49	20.83 ± 0.42	20.77 ± 0.38	20.67 ± 0.47	20.57 ± 0.50	20.53 ± 0.51
Turmeric	P_1	21.17 ± 0.78	21.17 ± 0.68	21.17 ± 0.67	21.17 ± 0.75	21.17 ± 0.67	21.17 ± 0.78	21.13 ± 0.57
	P_2	21.17 ± 0.78	21.17 ± 0.68	21.17 ± 0.67	21.17 ± 0.75	21.17 ± 0.67	21.17 ± 0.78	21.13 ± 0.57
Synthetic preservatives								
Acetic acid	P_1	20.93 ± 0.40	20.07 ± 0.06	19.20 ± 0.70	18.73 ± 0.46	17.63 ± 0.21	16.10 ± 0.36	14.07 ± 0.45
	P_2	20.93 ± 0.40	20.47 ± 0.72	19.33 ± 0.47	19.10 ± 0.30	17.83 ± 0.06	16.27 ± 0.49	14.30 ± 0.46
Potassium Sorbate	P_1	20.97 ± 0.76	20.23 ± 1.03	20.23 ± 1.03	20.23 ± 0.45	20.23 ± 0.45	20.13 ± 0.21	20.13 ± 0.06
	P_2	20.97 ± 0.76	20.23 ± 1.03	20.23 ± 1.03	20.23 ± 0.45	20.23 ± 0.15	20.17 ± 0.25	20.17 ± 0.06
Sodium Nitrite	P_1	20.97 ± 0.76	20.93 ± 0.49	20.93 ± 0.49	20.93 ± 0.49	20.90 ± 0.53	20.90 ± 0.52	20.90 ± 0.46
	P_2	20.97 ± 0.76	20.93 ± 0.49	20.93 ± 0.59	20.93 ± 0.51	20.93 ± 0.51	20.90 ± 0.52	20.90 ± 0.53

The data represent the mean ± SD of three replicates; ANOVA Table A.2 is given in annexure; P_1 =HDPE jar, P_2 =Glass jar
Treatment CD at 5% = P_1 (0.030) ; P_2 (0.035) ; Storage CD at 5% =P_1 (0.023) ; P_2 (0.027)

The change in protein content is attributed to significant lowering of the pH of meat pickle upon storage. The lower pH denatures the meat proteins, which results in structural alterations and coagulations owing to the unfolding of polypeptide chain. (Bacus,1986). As the denaturation process continues during storage, the unfolding exposes the side chains. Intramolecular affinity and binding yields the aggregates of varying size. The denaturation and coagulation of meat proteins also contributes to the moisture loss and discolouration of meat. Gill (1986) has also reported the discolouration upon denaturation of protein and the colour of meat changes to brown at pH 4.5. Indeed, the proteolysis of protein constitutes the main biochemical reaction in the generation of meat flavour (Toldra, 1998). Besides, pH, the temperature, interface area, organic solvents, salts, etc. are other factors causing denaturation of protein (Springer, 1999). Some of these might be contributing in the observed changes in protein contents of meat pickles during pickling and storage processes.

4.1.1.3 TBA Number

TBA number of 1.0 mg / kg is reported as a threshold level of lipid rancidity. The raw buffalo meat exhibits the TBA value of 0.3 mg/kg. The data in Table 4.3 show that the TBA values of meat pickles, ranges between 0.26 to 0.31. The results indicate both the qualitative and quantitative changes in the chemical nature of meat fat in control pickle. The TBA values in control also increased substantially as a function of time. However, the TBA value of 0.79 in HDPE jar and 0.57 in glass jar after 120 days storage at room temperature is still much lower than the threshold limit (1mg/kg) of rancidity. The TBA values of meat pickles preserved in natural and synthetic preservatives have been found to be low. Moreover, no significant increases in TBA number has been noticed as a function of time in meat pickle treated with natural preservatives. On the contrary, the TBA values showed slight increase in meat pickle with synthetic preservatives. The data show that the TBA decreases

Table 4.3 Effect of treatments on TBA numbers of buffalo meat pickles during storage of 120 days at ambient temperature.

Samples		TBA numbers (mg/kg) as a function of storage time (days)						
		0	20	40	60	80	100	120
Control (untreated)	P_1	0.31 ± 0.01	0.33 ± 0.02	0.40 ± 0.06	0.46 ± 0.09	0.52 ± 0.11	0.56 ± 0.11	0.79 ± 0.01
	P_2	0.31 ± 0.01	0.30 ± 0.02	0.35 ± 0.06	0.37 ± 0.05	0.35 ± 0.01	0.53 ± 0.06	0.57 ± 0.09
Natural products								
Cinnamon	P_1	0.29 ± 0.02	0.26 ± 0.05	0.26 ± 0.05	0.25 ± 0.06	0.24 ± 0.03	0.26 ± 0.04	0.28 ± 0.02
	P_2	0.29 ± 0.02	0.25 ± 0.05	0.24 ± 0.04	0.24 ± 0.06	0.24 ± 0.03	0.24 ± 0.06	0.25 ± 0.06
Clove	P_1	0.26 ± 0.03	0.24 ± 0.06	0.20 ± 0.07	0.19 ± 0.05	0.19 ± 0.03	0.19 ± 0.04	0.19 ± 0.04
	P_2	0.26 ± 0.03	0.23 ± 0.08	0.18 ± 0.05	0.16 ± 0.02	0.16 ± 0.02	0.16 ± 0.02	0.16 ± 0.05
Garlic	P_1	0.28 ± 0.01	0.26 ± 0.05	0.26 ± 0.05	0.27 ± 0.05	0.32 ± 0.02	0.34 ± 0.04	0.40 ± 0.05
	P_2	0.28 ± 0.01	0.24 ± 0.03	0.24 ± 0.04	0.25 ± 0.03	0.29 ± 0.04	0.31 ± 0.02	0.35 ± 0.04
Mustard	P_1	0.29 ± 0.03	0.25 ± 0.06	0.26 ± 0.07	0.28 ± 0.05	0.28 ± 0.03	0.29 ± 0.02	0.33 ± 0.02
	P_2	0.29 ± 0.03	0.25 ± 0.06	0.26 ± 0.07	0.28 ± 0.05	0.28 ± 0.03	0.28 ± 0.02	0.28 ± 0.02
Turmeric	P_1	0.28 ± 0.01	0.28 ± 0.04	0.21 ± 0.06	0.09 ± 0.05	0.08 ± 0.02	0.14 ± 0.04	0.19 ± 0.03
	P_2	0.28 ± 0.01	0.26 ± 0.05	0.19 ± 0.05	0.08 ± 0.04	0.07 ± 0.02	0.12 ± 0.01	0.17 ± 0.03
Synthetic preservatives								
Acetic acid	P_1	0.28 ± 0.06	0.09 ± 0.02	0.07 ± 0.06	0.16 ± 0.03	0.24 ± 0.03	0.21 ± 0.05	0.19 ± 0.02
	P_2	0.28 ± 0.06	0.09 ± 0.01	0.08 ± 0.06	0.14 ± 0.01	0.22 ± 0.01	0.29 ± 0.07	0.17 ± 0.03
Potassium Sorbate	P_1	0.31 ± 0.01	0.24 ± 0.02	0.23 ± 0.02	0.23 ± 0.02	0.38 ± 0.03	0.44 ± 0.06	0.51 ± 0.01
	P_2	0.31 ± 0.01	0.28 ± 0.06	0.28 ± 0.06	0.30 ± 0.03	0.35 ± 0.01	0.45 ± 0.02	0.46 ± 0.01
Sodium Nitrite	P_1	0.28 ± 0.06	0.29 ± 0.02	0.29 ± 0.07	0.31 ± 0.04	0.46 ± 0.01	0.48 ± 0.03	0.51 ± 0.01
	P_2	0.28 ± 0.06	0.28 ± 0.06	0.28 ± 0.06	0.30 ± 0.03	0.35 ± 0.01	0.42 ± 0.06	0.46 ± 0.01

The data represent the mean ± SD of three replicates; ANOVA Table A.3 is given in annexure; P_1 =HDPE jar, P_2 =Glass jar
Treatment CD at 5% = P_1 (0.05) ; P_2 (0.08) ; Storage CD at 5% =P_1 (0.08) ; P_2 (0.06)

significantly after 20 to 40 days of storage then increases again to a maximum value of 0.19 and 0.17 mg/kg in HDPE and glass jar, respectively. On day 1, the meat pickles invariably exhibited the TBA values ranging from 0.26 to 0.29 mg /kg. These value are insignificantly ($P>0.05$) lower than the TBA value of raw meat (0.3) or control samples (0.31mg/kg). The data suggest that in general the TBA number of meat does not change except in case of synthetic preservatives.

Furthermore, the data revealed that the storage of pickles at ambient temperature for 120 days exerts varied effects on TBA values. Significant ($P<0.05$) increase in TBA values with increased storage period have been observed in case of control, garlic, potassium sorbate and sodium nitrite treated pickles while significant decrease ($P<0.05$) in TBA values of pickles treated with clove, turmeric and acetic acid were observed. In case of treatments with cinnamon and mustard, the insignificant ($P>0.05$) changes occurred in TBA values. In all cases, the TBA values of pickles packed in HDPE jars were found to be relatively higher as compared to glass jars. The difference in TBA values of pickles packed in HDPE jar as compared to glass jar was found to be significant ($P<0.05$) in case of control. Das (2002) also showed that the TBA values of acetic acid, potassium sorbate, and ascorbic acid + potassium sorbate + sodium nitrite and citric acid + potassium sorbate + sodium nitrite treated chevon meat increases significantly ($P<0.01$) during storage in both the polypropylene and paper aluminum-foil polythene. Grugic and Mulalic (1992) reported that an increase in TBA value during storage did not affect the sensory score of rancidity. Increase in TBA value is associated with progressive lipid oxidation and proteolytic changes. Lipid oxidation and pigment oxidation are auto catalytic and each process encourages the other.

Overall the data suggested that the treatment of meat pickles with spices prevent rancidity and act as antioxidants. Moreover, the salts promote the chemical

changes in meat and meat pigment because of which meat colour changes indicative of rancidity effect.

4.1.1.4. Ash content

Ash content of a food product represents the availability of minerals. The reported ash content of raw buffalo meat is 1.0 %. In comparison to raw meat, the ash contents of meat pickles have increased significantly ($P<0.05$) and vary in the range from 1.97 to 2.09 %, exhibiting a significant effect of pickling treatments (Table 4.4). Maximum increase in ash content of pickled meat as compared to raw meat was observed in case of sodium nitrite treatment followed by other treatments in order as; sodium nitrite > turmeric = mustard > cinnamon > clove = potassium sorbate = acetic acid > garlic. The variations in ash contents is attributed to mineral contents of various pickling treatments and ingredients.

Insignificant effect of storage period on the ash content of pickles was observed. Also, the changes in fat and protein contents of pickles during storage did not significantly ($P<0.05$) affect the ash content of pickles. These results are in accordance with studies of Ziauddin et al. (1994), who have reported that the protein and fat did not affect the ash content of meat and meat products. The packaging materials (HDPE and glass jars) also did not influenced the ash contents of stored meat pickles.

4.1.2 Minimum inhibitory concentration (MIC) of natural and synthetic preservatives used as treatments.

The Tables 4.5 and 4.6 show the minimum inhibition concentrations of both the natural and synthetic preservatives. The MIC was determined with four pure cultures of bacterial strains commonly associated with meat and meat products spoilage, including two Gram positive (*Staphylococcus aureus*, *Listeria monocytogenes*) and two Gram negative species (*Escherichia coli* and *Salmonella*

Table 4 .4 Effect of treatments on ash content of buffalo meat pickles during storage of 120 days at ambient temperature.

Samples		Ash content (%) as a function of storage time (days)						
		0	20	40	60	80	100	120
Control (untreated)	P_1	2.03 ± 0.06	2.03 ± 0.06	2.03 ± 0.06	2.06 ± 0.12	2.06 ± 0.12	2.06 ± 0.12	2.06 ± 0.12
	P_2	2.03 ± 0.06	2.03 ± 0.06	2.03 ± 0.06	2.06 ± 0.12	2.06 ± 0.12	2.06 ± 0.12	2.06 ± 0.12
Natural products								
Cinnamon	P_1	2.00 ± 0.10	2.00 ± 0.00	2.00 ± 0.10	2.00 ± 0.10	2.00 ± 0.10	2.03 ± 0.12	2.03 ± 0.12
	P_2	2.00 ± 0.00	2.00 ± 0.00	2.00 ± 0.00	2.00 ± 0.10	2.03 ± 0.12	2.03 ± 0.12	2.03 ± 0.12
Clove	P_1	1.98 ± 0.02	1.98 ± 0.01	1.98 ± 0.02	1.99 ± 0.01	1.99 ± 0.01	1.99 ± 0.01	1.99 ± 0.01
	P_2	1.98 ± 0.02	1.98 ± 0.02	1.98 ± 0.02	1.98 ± 0.02	1.99 ± 0.01	1.99 ± 0.01	1.99 ± 0.01
Garlic	P_1	1.97 ± 0.20	1.97 ± 0.10	1.97 ± 0.12	1.97 ± 0.15	1.97 ± 0.17	1.97 ± 0.10	1.97 ± 0.15
	P_2	1.97 ± 0.12	1.97 ± 0.12	1.97 ± 0.12	1.97 ± 0.12	1.97 ± 0.12	1.97 ± 0.12	1.97 ± 0.12
Mustard	P_1	2.03 ±0.20	2.03 ± 0.10	2.02 ± 0.07	2.02 ± 0.07	2.03 ± 0.15	2.02 ± 0.17	2.02 ± 0.17
	P_2	2.03 ±0.20	2.03 ± 0.10	2.03 ± 0.10	2.03 ± 0.10	2.02 ± 0.07	2.02 ± 0.15	2.02 ± 0.15
Turmeric	P_1	2.03 ± 0.06	2.03 ± 0.06	2.03 ± 0.12	2.03 ± 0.06	2.03 ± 0.12	2.03 ± 0.06	2.07 ± 0.06
	P_2	2.03 ± 0.06	2.03 ± 0.06	2.03 ± 0.06	2.03 ± 0.12	2.03 ± 0.12	2.03 ± 0.12	2.07 ± 0.06
Synthetic preservatives								
Acetic acid	P_1	1.98 ± 0.02	1.98 ± 0.02	1.98 ± 0.02	1.98 ± 0.02	1.99 ± 0.01	1.99 ± 0.01	1.99 ± 0.01
	P_2	1.98 ± 0.02	1.98 ± 0.02	1.98 ± 0.02	1.98 ± 0.02	1.99 ± 0.01	1.99 ± 0.01	1.99 ± 0.01
Potassium Sorbate	P_1	1.98 ± 0.02	1.98 ± 0.02	1.98 ± 0.01	1.99 ± 0.01	1.99 ± 0.01	1.99 ± 0.01	1.99 ± 0.01
	P_2	1.98 ± 0.02	1.98 ± 0.02	1.98 ± 0.02	1.99 ± 0.01	1.99 ± 0.01	1.99 ± 0.01	1.99 ± 0.01
Sodium Nitrite	P_1	2.09 ± 0.11	2.09 ± 0.11	2.09 ± 0.12	2.09 ± 0.12	2.12 ± 0.17	2.13 ± 0.16	2.13 ± 0.16
	P_2	2.09 ± 0.11	2.09 ± 0.12	2.09 ± 0.12	2.09 ± 0.11	2.12 ± 0.17	2.13 ± 0.16	2.13 ± 0.16

The data represent the mean ± SD of three replicates; ANOVA Table A.4 is given in annexure; P_1 =HDPE jar, P_2 =Glass jar
Treatment CD at 5% = P_1 (0.04) ; P_2 (0.04) ; Storage CD at 5% =P_1 (0.04) ; P_2 (0.04)

Table 4.5 Minimum inhibitory concentration (MIC) of some natural products against specific bacterial indicators of meat spoilage

Natural products / Indicators strains	**Aqueous extracts (%)**				
	0.5	**1.0**	**2.0**	**4.0**	**5.0**
Cinnamon					
Staphylococcus aureus	-	-	+	++	+++
Salmonella enteritidis	-	-	+	++	+++
Escherichia coli		+	++	+++	++++
Listeria monocytogenes	-	-	-	+	++
Clove					
Staphylococcus aureus	-	+	++	+++	++++
Salmonella enteritidis	-	-	+	++	+++
Escherichia coli	-	-	+	++	+++
Listeria monocytogenes	-	+	++	+++	+++
Turmeric					
Staphylococcus aureus	-	+	++	+++	++++
Salmonella enteritidis	-	-	+	++	+++
Escherichia coli	-	-	+	++	+++
Listeria monocytogenes	-	-	+	++	+++
Garlic					
Staphylococcus aureus	+	++	+++	++++	++++
Salmonella enteritidis	-	-	+	++	+++
Escherichia coli	-	-	+	++	+++
Listeria monocytogenes	-	-	-	+	++
Mustard					
Staphylococcus aureus	-	+	++	+++	+++
Salmonella enteritidis	-	-	+	++	+++
Escherichia coli	-	-	+	++	+++
Listeria monocytogenes	-	-	-	-	+

-:0 mm, + : 5-10 mm, ++ : 10-15 mm, +++ : 15-20 mm, ++++ : > 20 mm

Table 4.6 Minimum inhibitory concentration (MIC) of synthetic preservatives used against specific bacterial indicators of meat spoilage

Preservatives / Indicators strains	**Concentration of preservatives (%)**				
	0.010	**0.015**	**0.020**	**0.025**	**0.030**
Sodium nitrite					
Staphylococcus aureus	-	-	+	++	+++
Salmonella enteritidis	-	-	+	++	+++
Escherichia coli		+	++	+++	++++
Listeria monocytogenes	-	-	+	++	+++
Potassium sorbate					
Staphylococcus aureus	-	-	-	+	++
Salmonella enteritidis	-	-	-	+	++
Escherichia coli Type	-	-	-	+	++
Listeria monocytogenes	-	-	-	-	+
	0.5	**1.0**	**2.0**	**4.0**	**6.0**	**8.0**
Acetic acid						
Staphylococcus aureus	-	-	-	-	+	++
Salmonella enteritidis	-	-	-	-	+	++
Escherichia coli	-	-	-	+	++	+++
Listeria monocytogenes	-	-	-	-	+	++
				-	-	+

-:0 mm, + : 5-10 mm, ++ : 10-15 mm, +++ : 15-20 mm, ++++ : > 20 mm

enteritidis). The data suggested that 2% extracts of preservatives could significantly inhibit the growth of these organisms on nutrient agar medium. However, with the synthetic preservative, the MIC of sodium nitrite, potassium sorbate and acetic acid were determined to be 0.02% , 0.025% and 6%, respectively.

4.1.3. Microbiological characteristics

The microbiological quality of meat pickles has been assessed by determining the total plate count (TPC), as well as the proteolytic, lipolytic, coliform, *Staphylococcus*, yeast and mold (Y&M) counts. The microbial profiles of meat pickles are shown in Tables 4.7 to 4.12.

4.1.3.1. Total plate counts (TPC)

The influence of various natural and chemical preservatives on the total plate counts (TPC) was determined. The data shown in Table 4.7 revealed that both the treatment doses and storage conditions exert significant impact ($P<0.05$) on the microbial quality of meat in pickles. During 120 days of storage, the TPC value of control sample of meat pickle increased significantly ($P<0.05$) with increasing period of storage. The initial total plate count of the control sample was determined to be 4.73 x 10^3 CFU/g, which increased upon storage to 11.8 x 10^3 CFU/g in HDPE and 10.8 x 10^3 CFU/g in glass jars, on 120^{th} day. The meat pickles even after 120 days of storage at ambient temperature were in edible condition. This corroborates well with the results of Yadav et al. (2003), who has also reported similar observations on quail egg pickle.

Comparative analysis of meat pickle (control verses treated and with in treatment groups) showed reduction in microbial population as a function of storage time with almost all the treatments, irrespective of the packaging material. Amongst the natural products used in this study, the effect of 2% clove on microbial control was prominent. This spice caused significant decrease ($P < 0.05$) in bacterial

Table-4-7 Effect of treatments on TPC in buffalo meat pickles during storage of 120 days at ambient temperature.

Samples		TPC ($x10^3$CFU/g) as a function of storage time (days)						
		0	**20**	**40**	**60**	**80**	**100**	**120**
Control (untreated)	P_1	4.73 ± 0.16	5.17 ± 0.64	6.17 ± 0.71	6.70 ± 0.70	6.33 ± 0.21	9.77 ± 0.60	11.8 ± 1.22
	P_2	4.73 ± 0.16	4.93 ± 0.06	5.83 ± 0.45	6.37 ± 0.15	6.00 ± 0.46	8.70 ± 0.62	10.8 ± 0.53
Natural products								
Cinnamon	P_1	3.33 ± 0.46	3.33 ± 0.12	2.47 ± 0.51	2.10 ± 0.10	1.70 ± 1.25	1.07 ± 0.51	0.67 ± 0.12
	P_2	3.33 ± 0.46	2.87 ± 0.32	2.10 ± 0.27	1.60 ± 0.46	0.73 ± 0.40	ND	ND
Clove	P_1	3.17 ± 0.12	2.07 ± 0.64	1.67 ± 0.25	0.43 ± 0.21	ND	ND	ND
	P_2	3.17 ± 0.12	1.73 ± 0.15	1.13 ± 0.51	ND	ND	ND	ND
Garlic	P_1	3.66 ± 0.35	3.26 ± 0.84	2.73 ± 0.15	2.13 ± 0.15	1.03 ± 0.70	0.67 ± 0.15	0.37 ± 0.16
	P_2	3.66 ± 0.35	3.66 ± 0.45	1.93 ± 0.59	1.07 ± 0.59	0.37 ± 0.31	ND	ND
Mustard	P_1	3.50 ± 0.52	3.17 ± 0.51	2.90 ± 1.25	2.77 ± 1.10	2.60 ± 0.99	2.90 ± 0.44	3.27 ± 0.32
	P_2	3.50 ± 0.52	3.07 ± 0.45	2.47 ± 0.95	2.43 ± 0.90	2.47 ± 0.95	2.27 ± 0.76	2.53 ± 0.21
Turmeric	P_1	3.60 ± 0.10	3.17 ± 0.51	2.13 ± 0.40	1.77 ± 0.59	1.53 ± 0.67	1.13 ± 0.35	0.67 ± 0.16
	P_2	3.60 ± 0.10	2.73 ± 0.31	1.57 ± 0.22	1.77 ± 0.56	1.27 ± 0.28	ND	ND
Synthetic preservatives								
Acetic acid	P_1	3.23 ± 0.40	1.53 ± 0.06	1.23 ± 0.15	0.77 ± 0.64	ND	ND	ND
	P_2	3.23 ± 0.40	1.17 ± 0.06	ND	ND	ND	ND	ND
Potassium Sorbate	P_1	3.87 ± 0.40	3.93 ± 0.35	4.17 ± 0.71	4.50 ± 0.61	5.07 ± 0.40	5.83 ± 0.45	6.67 ± 0.23
	P_2	3.87 ± 0.40	3.83 ± 0.40	4.10 ± 0.62	4.40 ± 0.53	4.93 ± 0.29	5.77 ± 0.47	6.60 ± 0.20
Sodium Nitrite	P_1	3.67 ± 0.23	ND	ND	ND	ND	ND	1.23 ± 0.06
	P_2	3.67 ± 0.23	ND	ND	ND	ND	ND	1.17 ± 0.06

The data represent the mean ± SD of three replicates; ANOVA Table A.5 is given in annexure; P_1 =HDPE jar, P_2 =Glass jar
Treatment CD at 5% = P_1 (0.25) ; P_2 (0.21) ; Storage CD at 5% =P_1 (0.22) ; P_2 (0.18); ND ; not detected

counts, and almost no growth was observed after 80 days of storage. Clove is reported to have antibacterial and yeast inhibitory activity (Sirnik and Gorisek, 1983 ; Suresh et al., 1992) and antimycotic activity (Azzouz and Bullerman,1982 ; Muramatsu et al., 1998). The active principle that exhibit these properties of clove has been identified as 2 methoxy-4–2– propenol (Beuchat and Golden,1989). Besides, the clove also contains 15.4% essential oils, which has 92% eugenol (Hitokoto et al.,1980). In addition to the suppression of bacterial growth as demonstrated in this study, the 2% concentration of clove has also been reported to completely inhibit fungal growth in culture. Out of all five natural products viz. cinnamon, clove, turmeric, garlic and mustard tested in this study, mustard was noticed to be least effective. Based on their microbial control potential, the efficacy of these natural products as preservatives in meat pickle was determined to be in the order as clove > garlic > turmeric = cinnamon > mustard.

The quality of pickle upon storage with the chemical preservatives viz. potassium sorbate, sodium nitrite and acetic acid has also been assessed and data presented in Table 4.7. The results demonstrated the efficacy of chemical treatments in the order as sodium nitrite > acetic acid > potassium sorbate. In general, all the three treatments significantly ($P<0.05$) inhibited the microbial growth. In case of sodium nitrite treated meat pickles, the total plate count decreased significantly ($P<0.05$) and no microbial growth appeared after 20 days of storage. Nitrites in meat are known to produce nitrosamines, which inhibit the growth of bacteria (Arya, 2003). Similarly , the acetic acid also inhibits the microbial growth owing to protein denaturation caused due to low pH (Ray et al., 2003). Although, 0.2% potassium sorbate is commonly used as a preservative, the addition of this preservatives to meat pickle at concentration of 0.025% showed a dismal effect on test bacteria. Nevertheless, the comparative analysis revealed that the storage of meat pickle in

glass jars is much safer than the HDPE jars for long-term storage under ambient conditions .

4.1.3.2. Coliform count

Table 4.8 presents the effect of natural products and chemical preservatives on coliform group of bacteria in meat pickle stored at ambient temperature. The data indicate low initial (1.7 x 10^3 CFU/g) counts of coliforms in control meat pickles. However, the population increased significantly to 5.6 and 4.1 x 10^3 CFU/g at the end of the storage period of 120 days in HDPE and glass jar, respectively. Although, high level of coliforms have been reported in buffalo carcasses slaughtered in traditional slaughter unit (Ziauddin et al., 1994), the population reduces substantially with successive treatments during cooking and food processing. Approximately, $5x10^6$ CFU/g aerobic microflora and 50 *E.coli* /g have been suggested as microbiological limits for fresh meat (Carl, 1975).

Treatment of meat pickles with natural and synthetic preservatives significantly ($P<0.05$) reduced the level of coliforms. The growth remains 100% suppressed till 120 days of storage invariably with all treatments under both the storage conditions. However, in case of control meat pickles stored in HDPE jar, the coliforms count was relatively higher vis-a-vis glass jars, which suggest the suitability of glass jar for long-term storage of meat pickles. Almost all the natural and chemical preservatives added to meat pickles showed inhibitory effect on coliforms except mustard and potassium sorbate. Meat pickles treated with mustard and potassium sorbate exhibited recurrence of *E.coli* incidence after 20 days of storage. Moreover, the population of bacteria increased with increasing storage time. The reason for higher total bacterial counts and coliforms in mustard treated meat pickles indicates the weak antimicrobial property of the preservative. This corroborates well with the reportedly poor bacteriostatic activity of mustard (Leuschner and Zamparini,

Table 4.8. Effect of treatments on coliform counts in buffalo meat pickles during storage of 120 days at ambient temperature.

Samples		Coliforms($x10^3$CFU/g) as a function of storage time (days)						
		0	20	40	60	80	100	120
Control (untreated)	P_1	ND	1.73 ± 0.16	2.33 ± 0.25	3.67 ± 0.81	4.67 ± 0.81	4.97 ± 0.71	5.63 ± 0.5
	P_2	ND	1.67 ± 0.16	1.83 ± 0.64	2.83 ± 0.46	3.57 ± 1.10	3.97 ± 1.00	4.17 ± 0.1
Natural products								
Cinnamon	P_1	ND	ND	ND	ND	ND	ND	ND
	P_2	ND	ND	ND	ND	ND	ND	ND
Clove	P_1	ND	ND	ND	ND	ND	ND	ND
	P_2	ND	ND	ND	ND	ND	ND	ND
Turmeric	P_1	ND	ND	ND	ND	ND	ND	ND
	P_2	ND	ND	ND	ND	ND	ND	ND
Garlic	P_1	ND	ND	ND	ND	ND	ND	ND
	P_2	ND	ND	ND	ND	ND	ND	ND
Mustard	P_1	ND	1.43 ± 0.21	1.43 ± 0.25	1.43 ± 0.31	1.67 ± 0.12	1.79 ± 0.17	1.83 ± 0.35
	P_2	ND	1.33 ± 0.21	1.33 ± 0.15	1.37 ± 0.31	1.53 ± 0.06	1.63 ± 0.16	2.33 ± 0.15
Synthetic preservatives								
Acetic acid	P_1	ND	ND	ND	ND	ND	ND	ND
	P_2	ND	ND	ND	ND	ND	ND	ND
Potassium sorbate	P_1	ND	1.37 ± 0.06	1.73 ± 0.06	1.77 ± 0.06	2.07 ± 0.25	2.17 ± 0.35	2.27 ± 0.40
	P_2	ND	1.27 ± 0.06	1.73 ± 0.06	1.77 ± 0.06	1.83 ± 0.70	1.87 ± 0.70	1.93 ± 0.75
Sodium nitrite	P_1	ND	ND	ND	ND	ND	ND	ND
	P_2	ND	ND	ND	ND	ND	ND	ND

The data represent the mean ± SD of three replicates; ANOVA Table A.6 is given in annexure; P_1 =HDPE jar, P_2 =Glass jar
Treatment CD at 5% = P_1 (0.11) ; P_2 (0.14) ; Storage CD at 5% =P_1 (0.09) ; P_2 (0.12); ND ; not detected

2002). Similarly, the growth inhibitory effect of garlic on coliforms is attributed to its antimicrobial activity. The antibacterial effect of garlic against *E.coli* and *Salmonella typhi* has been demonstrated (Ahmad et al., 1993 and Arora and Kaur, 1999).

Indeed, clove 2% was assessed to be the most effective antimicrobial preservative used in meat pickle. This observation is in accordance with earlier reported results of Leuschner and Zamparini (2002). Also, the effect of clove extract on the production of vero-toxin by enterohcmorrhagic *E.coli* (EHEC) 0157:H_7 has been reported (Sakagami et al., 2000). Moreover, Kumar and Berwal, (1998) demonstrated the sensitivity of food pathogens to spices. Leuschner and Zamparine (2002) have reported that 1% clove exhibit high bactericidal activity on both *Salmonella enteritidis* and *E.coli 0157*:H7. The antimicrobial activity of clove may be due to the active principle eugenol, which has been found to be effective against Gram-negative microorganisms such as *E.coli* (Suresh et al., 1992). Thus the application of these natural products to meat pickles not only add flavour to the product but also prolongs the shelf-life by reducing the load of microbial food pathogens and non pathogens.

4.1.3.3. *Staphylococcus* counts

Table 4.9 shows the extent of *Staphylococcal* contamination in control and treated meat pickles and the effect of storage time and conditions on *Staphylococcus* population. The untreated control meat pickle exhibited increase in bacterial count with the initial level of 1.53×10^3 to 4.07×10^3 and 3.67×10^3 upon 120 days storage in HDPE and glass jars, respectively. The reported maximum limit of *Staphylococcal* count in food is 10^5 CFU/g or ml (ISO, 2003). The anti-bacterial effect of spices treatments on meat pickle in this study is evident with the significant reduction ($P<0.05$) in the overall bacterial load during storage.

The natural products including clove, turmeric, garlic and mustard treated

Table 4.9 Effect of treatments on *Staphylococcus* counts in buffalo meat pickles during storage of 120 days at ambient temperature.

Samples		*Staphylococcus* (x10^3CFU/g) as a function of storage time (days)						
		0	20	40	60	80	100	120
Control (untreated)	P_1	1.53 ± 0.06	1.63 ± 0.40	2.27 ± 0.15	2.43 ± 0.06	2.77 ± 0.64	3.37 ± 0.61	4.07 ± 0.71
	P_2	1.53 ± 0.06	1.33 ± 0.21	1.73 ± 0.71	1.97 ± 0.51	2.13 ± 0.55	3.13 ± 0.57	3.67 ± 1.19
Natural products								
Cinnamon	P_1	1.33 ± 0.15	1.07 ± 0.57	0.43 ± 0.06	ND	ND	ND	ND
	P_2	1.33 ± 0.50	0.93 ± 0.43	0.53 ± 0.08	ND	ND	ND	ND
Clove	P_1	1.27 ± 0.12	ND	ND	ND	ND	ND	ND
	P_2	1.27 ± 0.12	ND	ND	ND	ND	ND	ND
Garlic	P_1	1.07 ± 0.06	ND	ND	ND	ND	ND	ND
	P_2	1.07 ± 0.06	ND	ND	ND	ND	ND	ND
Mustard	P_1	1.07 ± 0.12	ND	ND	ND	ND	ND	ND
	P_2	1.07 ± 0.12	ND	ND	ND	ND	ND	ND
Turmeric	P_1	1.07 ± 0.58	ND	ND	ND	ND	ND	ND
	P_2	1.07 ± 0.58	ND	ND	ND	ND	ND	ND
Synthetic preservatives								
Acetic acid	P_1	1.33 ± 0.15	1.27 ± 0.12	1.20 ± 0.00	1.17 ± 0.12	0.87 ± 0.58	0.73 ± 0.64	0.57 ± 0.06
	P_2	1.33 ± 0.15	1.27 ± 0.12	1.23 ± 0.06	1.03 ± 0.47	0.37 ± 0.23	ND	ND
Potassium Sorbate	P_1	1.17 ± 0.12	1.23 ± 0.12	1.27 ± 0.15	1.33 ± 0.12	1.43 ± 0.06	1.53 ± 0.06	1.67 ± 0.15
	P_2	1.17 ± 0.12	1.17 ± 0.12	1.17 ± 0.11	1.23 ± 0.12	1.27 ± 0.12	1.33 ± 0.15	1.47 ± 0.31
Sodium Nitrite	P_1	0.73 ± 0.64	ND	ND	ND	ND	ND	ND
	P_2	0.73 ± 0.64	ND	ND	ND	ND	ND	ND

The data represent the mean ± SD of three replicates; ANOVA Table A.7 is given in annexure; P_1 =HDPE jar, P_2 =Glass jar
Treatment CD at 5% = P_1 (0.14) ; P_2 (0.15) ; Storage CD at 5% =P_1 (0.12) ; P_2 (0.13) ; ND; not detected

meat pickles exhibited significant ($P<0.05$) decrease in *Staphylococcal* count after 20th day of storage. This is attributed to the active principles viz. eugenol, curcumin, allium and allyl-isothiocynate in these spices, which are effective against these bacteria. Chopra et al. (1941) have reported that curcumin and other curcuminoids also inhibit the growth of *S. albus* and *S.aureus.* Moreover, allicin, the chief constituent of garlic has also been shown to inhibit the growth of *S.aureus* (Powar et al., 1975). Similarly, Gocho (2000) stated that the clove and cinnamon both have the ability to stop the growth of *S. aureus*.

Amongst the chemically preserved meat pickle, potassium sorbate treated pickle showed the initial *Staphylococcal* count of 1.17 x 10^3 CFU/g, which marginally increased to 1.67 x 10^3 CFU/g and 1.47 x 10^3 CFU/g in HDPE and glass jar, respectively. However, the treatments with sodium nitrite followed by acetic acid have suppressed the *Staphylococcus* count after 20 and 100 days of storage. In case of acetic acid treated meat pickle, a gradual reduction in *Staphylococcal* count was noticed. The effect of acetic acid was more pronounced in case of meat pickle stored in glass jars. Thus, the packaging materials also exert some protective effect on the microbial quality of the stored meat pickles.

4.1.3.4.. Proteolytic and lipolytic counts

The presence of proteolytic and lipolytic bacteria in meat pickle and the effect of storage time and conditions have been assessed. The results shown in Table 4.10 indicate significant population of proteolytic bacteria, on day 1 in control untreated meat pickle. The proteolytic counts increased with storage time under ambient temperature. In contrast, the lipolytic bacteria initially absent upon 20 days of storage and sustained with marginal increase in population during storage upto 120 days (Table 4.11). However, treatment of meat pickle with clove and turmeric resulted in 100% growth inhibition of both the proteolytic and lipolytic microorganisms.

Table 4.10 Effect of treatments on proteolytic count in buffalo meat pickles during storage of 120 days at ambient temperature.

Samples		Proteolytic ($x10^3$CFU/g) as a function of storage time (days)						
		0	20	40	60	80	100	120
Control (untreated)	P_1	1.47 ± 0.06	1.63 ± 0.15	1.73 ± 0.50	2.03 ± 0.64	2.13 ± 0.67	2.27 ± 0.51	2.63 ± 0.15
	P_2	1.47 ± 0.06	1.43 ± 0.32	1.57 ± 0.57	1.73 ± 0.40	2.16 ± 0.35	2.53 ± 0.64	2.97 ± 0.60
Natural products								
Cinnamon	P_1	0.93 ± 0.32	0.73 ± 0.16	ND	ND	ND	ND	ND
	P_2	0.93 ± 0.32	0.67 ± 0.15	ND	ND	ND	ND	ND
Clove	P_1	ND	ND	ND	ND	ND	ND	ND
	P_2	ND	ND	ND	ND	ND	ND	ND
Garlic	P_1	1.23 ± 0.23	0.87 ± 0.78	0.90 ± 0.79	0.93 ± 0.81	1.43 ± 0.25	1.57 ± 0.12	1.63 ± 0.50
	P_2	1.23 ± 0.23	0.80 ± 0.70	0.70 ± 0.61	0.77 ± 0.67	1.37 ± 0.15	1.50 ± 0.00	1.57 ± 0.50
Mustard	P_1	1.17 ± 0.65	ND	ND	1.23 ± 0.15	1.27 ± 0.06	1.33 ± 0.06	1.37 ± 0.15
	P_2	1.17 ± 0.65	ND	ND	1.03 ± 0.47	1.13 ± 0.29	1.17 ± 0.59	1.23 ± 0.45
Turmeric	P_1	ND	ND	ND	ND	ND	ND	ND
	P_2	ND	ND	ND	ND	ND	ND	ND
Synthetic preservatives								
Acetic acid	P_1	1.27 ± 0.06	1.17 ± 0.06	0.97 ± 0.23	0.57 ± 0.06	ND	ND	ND
	P_2	1.27 ± 0.06	1.03 ± 0.12	0.47 ± 0.06	ND	ND	ND	ND
Potassium Sorbate	P_1	ND	ND	ND	ND	ND	1.43 ± 0.21	1.53 ± 0.49
	P_2	ND	ND	ND	ND	ND	ND	1.37 ± 0.21
Sodium Nitrite	P_1	ND	ND	ND	ND	ND	1.13 ± 0.06	1.17 ± 0.06
	P_2	ND	ND	ND	ND	ND	ND	ND

The data represent the mean ± SD of three replicates; ANOVA Table A.8 is given in annexure; P_1 =HDPE jar, P_2 =Glass jar
Treatment CD at 5% = P_1 (0.13) ; P_2 (0.12) ; Storage CD at 5% =P_1 (0.12) ; P_2 (0.10) ; ND; not detected

Table 4.11 Effect of treatments on lipolytic count in buffalo meat pickles during storage of 120 days at ambient temperature.

Samples		Lipolytic (x10^3CFU/g) as a function of storage time (days)						
		0	20	40	60	80	100	120
Control (untreated)	P_1	ND	1.63 ± 0.06	1.97 ± 0.38	2.33 ± 0.25	2.37 ± 0.12	2.47 ± 0.23	2.62 ± 0.23
	P_2	ND	1.27 ± 0.21	1.57 ± 0.12	1.77 ± 0.35	1.83 ± 0.71	1.93 ± 0.76	1.97 ± 0.42
Natural products								
Cinnamon	P_1	ND	ND	ND	ND	ND	0.63 ± 0.15	1.13 ± 1.04
	P_2	ND	ND	ND	ND	ND	ND	0.97 ± 0.21
Clove	P_1	ND	ND	ND	ND	ND	ND	ND
	P_2	ND	ND	ND	ND	ND	ND	ND
Turmeric	P_1	ND	ND	ND	ND	ND	ND	ND
	P_2	ND	ND	ND	ND	ND	ND	ND
Garlic	P_1	ND	ND	ND	ND	ND	ND	ND
	P_2	ND	ND	ND	ND	ND	ND	ND
Mustard	P_1	ND	ND	ND	ND	ND	ND	ND
	P_2	ND	ND	ND	ND	ND	ND	ND
Synthetic preservatives								
Acetic acid	P_1	ND	ND	1.16 ± 0.06	1.56 ± 0.12	1.70 ± 0.27	2.47 ± 0.25	2.97 ± 0.29
	P_2	ND	ND	ND	ND	1.03 ± 0.78	1.17 ± 0.06	1.57 ± 0.12
Potassium Sorbate	P_1	ND	ND	1.17 ± 0.06	1.23 ± 0.67	1.27 ± 0.06	1.33 ± 0.67	1.47 ± 0.12
	P_2	ND	ND	ND	ND	1.07 ± 0.06	0.97 ± 0.42	1.17 ± 0.35
Sodium Nitrite	P_1	ND	ND	ND	ND	ND	1.13 ± 0.06	1.17 ± 0.06
	P_2	ND	ND	ND	ND	ND	ND	1.23 ± 0.23

The data represent the mean ± SD of three replicates; ANOVA Table A.9 is given in annexure; P_1 =HDPE jar, P_2 =Glass jar
Treatment CD at 5% = P_1 (0.08) ; P_2 (0.10) ; Storage CD at 5% =P_1 (0.07) ; P_2 (0.09) ; ND; not detected

Cinnamon as a natural product was effective in controlling the growth of proteolytic bacteria after 20 days of storage. Mustard and garlic were found to be least effective in checking the growth of proteolytic bacteria. On the contrary, all the natural products showed significant ($P<0.05$) bacteriostatic effect on lipolytic bacteria. Both the proteolysis and lypolysis in meat by microbial activity is influenced by storage temperature. It has been reported that maximum lipolytic and proteolytic activity occurs at lower temperatures. However, at temperature 30°C or above, these activities are severely inhibited. Predominantly, the proteolytic and lypolytic activities are the characters of psychrophiles. In meat the population of *Pseudomonas* sp. initially utilize the free amino acids and nucleotides and proteolysis of water soluble proteins occurs later. Our results indicate that storage of control meat pickle at ambient temperature facilitates the growth of proteolytic and lipolytic bacteria.

4.1.3.5.. Yeast and mold counts

In addition to bacterial counts, the yeast and molds have also been enumerated in stored meat pickles. The data presented in Table 4.12. indicate that storage of control untreated meat pickle under ambient condition favours the growth of yeasts and molds. The fungal population increases from an initial count of 2.73×10^3 to 11.2 and 9.27×10^3 in HDPE and glass jars respectively. Treatment of meat pickle with cinnamon and clove and turmeric resulted in significant ($P<0.05$) reduction in the fungal counts. Also, the acetic acid and sodium nitrite treatments inhibited the growth of fungi to a significant extent and no fungal count was noticed after 20 days of storage. Almost all treatments with natural preservatives, except garlic and mustard exhibited significant anti-fungal effect. The inhibitory effect of clove and potassium sorbate on fungal growth was very prominent. Clove contains 15.4% essential oils, which has 92% eugenol (Hitokoto et al.,1980). It has been reported that clove at 2% concentration completely inhibit *Aspergillus* and *Penicillium* species (Azzouz and

Table 4.12 Effect of treatments on Y&M counts in buffalo meat pickles during storage of 120 days at ambient temperature.

Samples		Y&M ($x10^3$CFU/g) as a function of storage time (days)						
		0	20	40	60	80	100	120
Control (untreated)	P_1	2.73 ± 0.49	3.57 ± 0.32	4.93 ± 0.51	6.83 ± 0.64	7.90 ± 0.46	8.83 ± 0.35	11.20 ± 0.64
	P_2	2.73 ± 0.49	3.23 ± 0.15	3.80 ± 0.52	4.93 ± 0.55	5.87 ± 0.72	7.17 ± 0.65	09.27 ± 0.57
Natural products								
Cinnamon	P_1	2.17 ± 0.12	1.93 ± 0.51	1.13 ± 0.06	ND	ND	ND	ND
	P_2	2.17 ± 0.12	0.73 ± 0.40	0.53 ± 0.38	ND	ND	ND	ND
Clove	P_1	1.50 ± 0.35	1.17 ± 0.55	ND	ND	ND	ND	ND
	P_2	1.50 ± 0.35	ND	ND	ND	ND	ND	ND
Turmeric	P_1	1.60 ± 0.46	1.33 ± 0.25	1.03 ± 0.67	0.73 ± 0.32	ND	ND	ND
	P_2	1.60 ± 0.46	1.07 ± 0.08	0.67 ± 0.36	ND	ND	ND	ND
Garlic	P_1	2.17 ± 0.12	4.17 ± 0.50	4.37 ± 0.46	5.60 ± 0.64	7.07 ± 0.64	9.03 ± 0.40	10.00 ± 0.40
	P_2	2.17 ± 0.12	3.83 ± 0.23	4.03 ± 0.57	5.50 ± 0.30	6.83 ± 0.25	8.03 ± 0.68	09.37 ± 0.75
Mustard	P_1	1.53 ± 0.15	1.77 ± 0.50	1.83 ± 0.40	1.87 ± 0.40	1.93 ± 0.61	1.97 ± 0.50	02.03 ± 0.40
	P_2	1.53 ± 0.15	1.57 ± 0.12	1.57 ± 0.06	1.63 ± 0.23	1.67 ± 0.46	1.73 ± 0.40	01.87 ± 0.29
Synthetic preservatives								
Acetic acid	P_1	ND	ND	ND	ND	ND	ND	ND
	P_2	ND	ND	ND	ND	ND	ND	ND
Potassium Sorbate	P_1	1.37 ± 0.15	1.03 ± 0.29	ND	ND	ND	ND	ND
	P_2	1.37 ± 0.15	N D	ND	ND	ND	ND	ND
Sodium Nitrite	P_1	1.36 ± 0.15	N D	ND	ND	1.13 ± 0.06	1.17 ± 0.06	01.40 ± 0.30
	P_2	1.36 ± 0.15	N D	ND	ND	ND	0.97 ± 0.25	01.27 ± 0.40

The data represent the mean ± SD of three replicates; ANOVA Table A.10 is given in annexure; P_1 =HDPE jar, P_2 =Glass jar
Treatment CD at 5% = P_1 (0.38) ; P_2 (0.19) ; Storage CD at 5% =P_1 (0.33) ; P_2 (0.17) ; ND; not detected

Bullerman, 1982). Moreover, Sharma et al. (1984) demonstrated the inhibitory activity of clove oil against aflatoxin producing *Aspergilli* .

Potassium sorbate is also a well known anti mycotic agent, which exhibits broad spectrum inhibitory effect against yeast and molds. Thus, the addition of potassium sorbate to meat pickles served as an important hurdle in controlling the fungal infection during storage at ambient temperature

Based on the data obtained from the bacteriological analysis of meat pickles, it is surmised that the application of spices at 2% concentration as one of the hurdle parameters preserved the meat in pickled form with minimal microbial load upon storage upto 120 days at ambient temperature. Besides, the spices as natural food additives contributed immensely to the taste and flavour of the meat pickle. These esoteric food adjuncts have been in use for thousand of years with established medicinal properties (Nadkarni and Nadkarni, 1976).

The extensive animal studies in the past several decades have established the role of spices as hypolipidimic agent (Srinivasan, 2004), antidiabetic agents (Srinivasan, 2004), digestion stimulant (Platel and Srinivasan, 2004), antioxidant and anti-inflammatory agents.

The roles of garlic and turmeric in management of diabetes mellitus, and lipid metabolism, and clove as anti-inflammatory agents, are well documented. Thus, enrichment of meat pickle with these spices adds medicinal value to meat pickle as a bonus. Majority of these spices added to the meat pickles are also known to stimulate pancreatic digestive enzymes such as lipases, amylases and proteases, which may play a crucial role in digestion. It has been reported that stimulation of bile secretion and activity of digestive enzymes by these spices, leads to accelerated digestion and reduction in food transit time in gastrointestinal tract.

Although, the spices were initially selected as a hurdle parameter to extend the shelf life of meat pickle, in view of their anti-microbial activity. However, the correlation of data with the properties of spices has unfolded the wealth of information. The analysis revealed that the supplementation of these traditional herbs to meat pickles exhibits multi pronged benefits. They may act as (i) anti-microbial preservatives, (ii) flavouring agent and (iii) a natural therapeutic agent with the host of beneficial physiological effects.

Spices do not contribute significantly to the nutrient makeup of the pickles. However, in view of many promising health beneficial physiological effects, these food adjuncts could be regarded as 'Nutraceuticals'. Therefore, it is concluded that the consumption of these spices in meat pickle may likely to make life not only more 'spicy' but more healthy too.

4.1.4 Organoleptic / sensory characteristics

Palatability of meat products depends upon the sensory characteristics like colour, odour, texture (tenderness), taste, etc. The data pertaining to some of these characteristics as sensory scores for various meat pickles are presented in Tables 4.13 to 4.21.

4.1.4.1 Colour

Treatments of meat with various pickling mediums consisting of different spices, chemicals and acids showed varying effects on the colour scores of the pickles (Table 4.13). The control samples on day 1 were rated as 'liked very much'. The only treatment, which enhanced the colour score of meat pickles on day 1, as compared to control samples, was with acetic acid. Other treatments, which decreased the colour scores of pickles, as compared to control samples on day 1, were in the decreasing order of turmeric/potassium sorbate > sodium nitrite > cinnamon or clove or garlic. Mustard treatment gave colour score equivalent to that of control treatment.

Table 4.13 Effect of treatments on colour score in buffalo meat pickles during storage of 120 days at ambient temperature.

Samples		Colour values as a function of storage time (days)						
		0	20	40	60	80	100	120
Control (untreated)	P_1	8.33 ± 1.16	8.33 ± 0.58	8.00 ± 1.00	8.00 ± 1.73	8.00 ± 0.00	7.67 ± 0.58	7.67 ± 0.58
	P_2	8.33 ± 1.16	8.33 ± 0.58	8.00 ± 1.00	8.00 ± 1.73	8.00 ± 0.00	7.67 ± 0.58	7.67 ± 1.16
Natural products								
Cinnamon	P_1	7.33 ± 0.58	6.67 ± 0.58	6.67 ± 0.58	6.33 ± 0.58	6.33 ± 1.53	6.33 ± 0.58	6.33 ± 0.58
	P_2	7.33 ± 0.58	7.00 ± 0.00	7.00 ± 0.00	6.67 ± 0.58	6.67 ± 0.58	6.67 ± 0.58	6.67 ± 0.58
Clove	P_1	7.33 ± 0.58	7.33 ± 0.58	7.33 ± 0.58	7.33 ± 0.58	7.33 ± 0.58	7.33 ± 0.58	7.00 ± 0.00
	P_2	7.33 ± 0.58	7.33 ± 1.15	7.33 ± 0.58	7.33 ± 1.15	7.33 ± 0.58	7.33 ± 1.15	7.33 ± 0.58
Garlic	P_1	7.33 ± 0.58	7.33 ± 1.53	7.33 ± 0.58	7.33 ± 0.58	7.33 ± 1.53	7.33 ± 0.58	7.33 ± 0.58
	P_2	7.33 ± 0.58	7.33 ± 1.16	7.33 ± 1.16	7.33 ± 1.53	7.33 ± 0.58	7.33 ± 0.58	7.33 ± 1.53
Mustard	P_1	8.33 ± 0.58	8.33 ± 0.58	8.33 ± 0.57	8.33 ± 0.58	8.00 ± 0.00	8.00 ± 0.00	8.00 ± 0.00
	P_2	8.33 ± 0.58	8.00 ± 1.73	8.33 ± 0.58	8.33 ± 0.58	8.33 ± 0.58	8.33 ± 0.58	8.33 ± 0.58
Turmeric	P_1	8.00 ± 1.00	8.00 ± 1.00	8.00 ± 1.73	8.00 ± 0.00	8.00 ± 1.73	8.00 ± 1.00	8.00 ± 1.73
	P_2	8.00 ± 1.00	8.00 ± 1.73	8.00 ± 1.00	8.00 ± 1.73	8.00 ± 1.73	8.00 ± 1.00	8.00 ± 1.73
Synthetic preservatives								
Acetic acid	P_1	8.67 ± 0.58	8.33 ± 0.58	8.00 ± 1.00	7.33 ± 1.16	7.00 ± 1.00	6.33 ± 0.58	5.67 ± 1.16
	P_2	8.67 ± 0.58	8.33 ± 0.58	8.33 ± 1.16	7.67 ± 1.53	7.33 ± 1.53	7.00 ± 1.00	6.00 ± 1.00
Potassium Sorbate	P_1	8.00 ± 1.00	8.00 ± 1.73	8.00 ± 1.00	8.00 ± 1.00	7.67 ± 1.53	7.67 ± 1.16	7.67 ± 0.58
	P_2	8.00 ± 1.00	8.00 ± 1.00	8.00 ± 0.00	8.00 ± 1.00	8.00 ± 1.73	7.67 ± 1.16	7.67 ± 0.58
Sodium Nitrite	P_1	7.67 ± 0.58	7.67 ± 1.53	7.67 ± 0.58	7.67 ± 0.58	7.67 ± 1.53	7.67 ± 1.16	7.67 ± 0.58
	P_2	7.67 ± 0.58	7.67 ± 1.53	7.67 ± 0.57	7.67 ± 0.58	7.67 ± 0.58	7.67 ± 1.53	7.67 ± 0.58

The data represent the mean ± SD of three replicates; ANOVA Table A.10 is given in annexure; P_1 =HDPE jar, P_2 =Glass jar
Treatment CD at 5% = P_1 (0.12) ; P_2 (0.14) ; Storage CD at 5% =P_1 (0.10) ; P_2 (0.10)

As may be noted from Table 4.13 the colour scores of fresh pickles, (a) treated separately with cinnamon, clove or garlic, (b) control samples or mustard treated samples, and (c) treated with potassium sorbate or turmeric within themselves differed insignificantly ($P>0.05$). The colour scores for all pickles on day 1 ranged between 7.33 to 8.67, showing their ranking between 'liked moderately' to 'liked very much'. Highest colour score of 8.67 on day 1 was awarded to acetic acid treated pickles followed by control and mustard treated samples. On the other hand, low colour scores were awarded to cinnamon, clove and garlic treated pickles.

Similarly, the packaging materials (HDPE and glass jar) and storage period (0 to 120 days) also exerted varying effects on colour scores of developed meat pickles. The effect of packaging materials and storage period on colour score was insignificant ($P>0.05$) in case of treatments with turmeric, garlic, clove and mustard for pickles preserved in glass jar, and with sodium nitrite for pickles preserved in both packaging materials.

Maximum and highly significant ($P<0.05$) decrease in colour score (defading of colour) was observed with storage time in case of acetic acid treated pickles. It was also noticed that glass jars are relatively better than HDPE jar with respect to colour stability of pickles.

Control samples and cinnamon, clove and, mustard (last two preserved in HDPE jar) and potassium sorbate treated pickles (preserved in packaging material) also showed defading of colour (reduction in colour score) during 120 days storage period. Highest colour score was recorded in case of mustard treated pickles (packed in glass jar) followed by turmeric treated pickles (packed in both packaging materials). The lowest colour score was recorded in case of acetic acid treated pickles packed in HDPE and glass jars.

Overall, the acetic acid treatment ranked superior on the day 1 but gradually developed unaccepted colour during storage. This may be due to excessive lipid oxidation, leading to drastic discolouration of meat pickle.

Other treatments, significantly ($P<0.05$) superior to all other treatments from the viewpoint of reduction in colour of pickles during ambient storage were with mustard and turmeric. Both of these natural products are known preservatives with typical yellow or orange brown food colour. Curcumin in turmeric and allyl-isothiocynate in mustard are the chief constituent of these spices, which may be responsible for typical yellow or orange brown colour. Potassium sorbate treated pickles also showed superior colour score on day 1, which, however, marginally reduced on completion of storage. Potassium sorbate treated pickles had a dull pale colour. The sodium nitrite treated pickles had static colour score during entire storage period. It is well known that nitrites react with meat pigments (myoglobin) to produce stable pigments, which are characteristics of cured meat.

Considering the extent of reduction in colour score during 120 days storage, the glass jar owing to the better barrier properties proved as a superior packaging material than HDPE for most of developed meat pickles.

The colour of developed meat pickles on day 1 as well as during storage had a strong correlation with their pH values (Table 4.13). It is well established that meat with high pH have better colour retention (Langlois, 1989). In the present study, the high pH values of pickles, on day 1, were of control samples followed by potassium sorbate, sodium nitrite, clove, cinnamon, mustard, garlic and turmeric treated pickles in decreasing order. Highest pH after 120 days storage was in case of pickles in following decreasing order; mustard treated pickles (packed in glass jar) and sodium nitrite treated pickles (packed in HDPE jar) > control samples (packed in glass jar) and mustard treated pickles (packed in HDPE jar) > turmeric treated pickles (packed

in glass jar) > turmeric treated pickles (packed in HDPE jar) > potassium sorbate treated pickles (packed in glass jar). The colour scores of pickles after 120 days of storage were more or less according to above changes in pH values. The pH of turmeric and mustard treated pickles showed lesser reduction with increase in storage period as compared to other treatments and accordingly had higher colour scores.

4.1.4.2.Odour

Odour expresses the aroma of food product. Formation of lipid break down products due to lipid oxidation in meat lead to development of undesirable flavour and odours. In this reference the odour score of developed meat pickles (Table 4.14), on day 1, in the range between 7 to 8.67, ranked all pickles between 'liked moderately' to 'liked very much' (closer to liked extremely). Pickling treatments with clove and mustard, in comparison to control samples, significantly ($P<0.05$) enhanced the odour score. On the contrary, the pickling treatments with cinnamon, turmeric and acetic acid significantly ($P<0.05$) reduced the odour score as compared to control. However, the odour scores were similar to control in pickles treated with garlic, potassium sorbate and sodium nitrite.

Highest odour scores were obtained in case of treatments with clove or mustard (individually) while lowest odour scores were obtained in case of treatments with turmeric or acetic acid. Eugenol, the active constituent present in clove and allyl-isothiocynate present in mustard may be the factors responsible for higher odour scores of pickles treated with these two spices. Similarly the aroma of curcumin present in turmeric, the phenols and aldehydes present in cinnamon and the unaccepted/unpleasant chemical odour of acetic acid contributed for reduced and/or poor odour scores of pickles treated with these spices.

In garlic, active constituent allium is the main flavouring agent, which imparts a strong, pervasive odour in addition to extremely pungent taste, liked by many

Table 4.14 Effect of treatments on odour score in buffalo meat pickles during storage of 120 days at ambient temperature.

Samples		Odour values as a function of storage time (days)						
		0	20	40	60	80	100	120
Control (untreated)	P_1	8.00 ± 1.00	8.67 ± 0.58	8.67 ± 0.58	8.67 ± 0.58	8.00 ± 1.00	7.33 ± 1.53	7.33 ± 1.53
	P_2	8.00 ± 1.00	8.67 ± 0.58	8.67 ± 0.58	8.67 ± 0.58	8.67 ± 0.58	8.67 ± 0.58	8.67 ± 0.58
Natural products								
Cinnamon	P_1	7.33 ± 1.16	7.00 ± 1.00	6.67 ± 1.53	6.67 ± 1.53	6.67 ± 1.16	6.33 ± 1.53	6.33 ± 1.53
	P_2	7.33 ± 1.16	7.00 ± 1.00	7.00 ± 0.00	7.00 ± 1.73	7.00 ± 2.00	7.00 ± 2.00	7.00 ± 2.00
Clove	P_1	8.67 ± 0.58	8.67 ± 0.58	8.67 ± 0.58	8.33 ± 0.58	8.33 ± 1.16	8.33 ± 1.16	8.33 ± 0.58
	P_2	8.67 ± 0.58	8.67 ± 0.58	8.67 ± 0.58	8.67 ± 0.58	8.67 ± 0.58	8.67 ± 0.58	8.67 ± 0.58
Garlic	P_1	8.00 ± 0.00	8.00 ± 1.73	8.00 ± 1.00	8.00 ± 1.00	7.67 ± 0.58	7.67 ± 1.53	7.67 ± 2.31
	P_2	8.00 ± 0.00	8.00 ± 1.73	8.00 ± 1.00	8.00 ± 1.73	8.00 ± 1.00	8.00 ± 1.00	8.00 ± 1.73
Mustard	P_1	8.67 ± 0.58	8.67 ± 0.58	8.67 ± 0.58	8.67 ± 0.58	8.33 ± 0.58	8.00 ± 1.00	8.33 ± 0.58
	P_2	8.67 ± 0.58	8.67 ± 0.58	8.67 ± 0.58	8.67 ± 0.58	8.67 ± 0.58	9.00 ± 0.00	9.00 ± 0.00
Turmeric	P_1	7.00 ± 0.00	7.00 ± 1.00	6.67 ± 1.53	6.67 ± 1.53	6.33 ± 0.58	6.33 ± 0.58	6.00 ± 1.00
	P_2	7.00 ± 0.00	7.00 ± 1.00	6.67 ± 0.58	6.67 ± 0.58	6.67 ± 1.16	6.67 ± 1.16	6.67 ± 1.16
Synthetic preservatives								
Acetic acid	P_1	7.00 ± 1.00	5.00 ± 1.00	5.00 ± 1.00	4.67 ± 0.58	4.33 ± 0.58	3.33 ± 1.16	3.00 ± 1.00
	P_2	7.00 ± 1.00	5.00 ± 1.00	5.00 ± 1.00	4.67 ± 0.58	4.33 ± 0.58	3.33 ± 1.16	3.00 ± 1.00
Potassium Sorbate	P_1	8.00 ± 1.00	8.33 ± 0.58	8.33 ± 0.58	8.33 ± 0.58	8.00 ± 1.00	8.00 ± 1.00	8.00 ± 1.00
	P_2	8.00 ± 1.00	8.00 ± 1.00	8.67 ± 0.58	8.67 ± 0.57	8.67 ± 0.58	8.67 ± 0.58	8.67 ± 0.58
Sodium Nitrite	P_1	8.00 ± 1.00	8.33 ± 0.58	8.33 ± 0.58	8.00 ± 1.00	7.67 ± 1.53	7.67 ± 1.53	7.67 ± 1.53
	P_2	8.00 ± 1.00	8.33 ± 0.58	8.33 ± 0.58	8.33 ± 1.16	8.67 ± 0.58	8.00 ± 1.73	8.67 ± 0.58

The data represent the mean ± SD of three replicates; ANOVA Table A.12 is given in annexure; P_1 =HDPE jar, P_2 =Glass jar
Treatment CD at 5% = P_1 (0.16) ; P_2 (0.14) ; Storage CD at 5% =P_1 (0.12) ; P_2 (0.11)

consumers. The thiosulfates of garlic are heat labile and disassociate on heating to form di and tri-sulphides and other sulphur compounds, which have been associated with cooked spice flavour.

Sodium nitrite, in addition to stabilizing the colour, also contributes to the characteristic flavour of cured meat. Potassium sorbate has also been traditionally used as a food preservative and is basically odourless.

The odour scores of pickles increased significantly ($P<0.05$) with increase in storage period control as well as in the mustard/ potassium sorbate/sodium nitrite treated pickles packed in glass jars. The glass jar, with better barrier properties, as compared to HDPE jar, proved superior packaging material for storage of meat pickles. However, the odour score decreased significantly ($P<0.05$) with increase in storage period in case of treatments with cinnamon, turmeric, and acetic acid (packed in both packaging materials), and clove, garlic, mustard, and sodium nitrite treated pickles and control samples (all packed in HDPE jar). These results suggest glass jars to be a better alternative to HDPE jar for controlling the odour of specific pickles during storage at ambient temperature. There was no change in odour score of pickles treated with clove and garlic (both packed in glass jar) and potassium sorbate (packed in HDPE jar) during 120 days of storage.

All other treatments except with acetic acid resulted in pickles which remained in good condition after 120 days storage with their odour scores ranking between minimum 'liked slightly' to maximum 'liked extremely'. The odour of only mustard treated pickles (packed in glass jar) was ranked 'liked extremely' at the end of 120 days of storage period. However, the odour of acetic acid treated pickles was significantly ($P<0.05$) non-acceptable.

4.1.4.3 Texture

Texture is another important characteristic, which expresses the compressibility and softness of the product. It is one of the palatability factors that determine the overall acceptability of the product. Table 4.15 presents the sensory texture scores of various types of meat pickles.

The texture score for control sample on day 1 was 5.33 indicating that the product was 'neither liked nor disliked'. The treatment with mustard was the only treatment giving texture score of pickles equivalent to control samples. Treatments with other preservatives like spices, salts and acetic acid resulted in low texture score in the following order: control > acetic acid > clove, or turmeric or sodium nitrite> cinnamon or potassium sorbate> garlic. This clearly suggests that treatments significantly ($P<0.05$) influenced the initial texture of pickles on day 1.The texture of pickles improved significantly upon storage on 120^{th} day of storage, the cinnamon, turmeric and mustard treated pickles exhibited the texture score of 8.33, equivalent to control suggesting that all the preserved pickles were 'liked very much'. On 120^{th} days of storage the pickles treated with clove and sodium nitrite also had the texture score of 8 ± 1.0 suggesting the placement of pickles in 'liked very much' category, though not to the same extent as of control samples.

Pickles treated with potassium sorbate and garlic were 'moderately liked' on the 120^{th} day of storage while acetic acid treated pickles were only 'slightly liked' as far as texture of pickles was concerned. Maximum improvement in texture of pickles during 120 days of storage was observed in case of treatment with cinnamon followed by other treatments in the decreasing order of turmeric > clove or sodium nitrite > control or garlic or mustard or potassium sorbate > acetic acid.

The improvement in tenderness of treated pickles during storage may be attributed to proteolysis of myofibrillar protein and control of lipid oxidation,

Table 4.15 Effect of treatments on texture score in buffalo meat pickles during storage of 120 days at ambient temperature.

Samples		Texture values as a function of storage time (days)						
		0	20	40	60	80	100	120
Control (untreated)	P_1	5.33 ± 0.58	6.67 ± 0.58	6.33 ± 0.58	7.00 ± 1.00	7.00 ± 1.00	7.67 ± 1.53	8.33 ± 0.58
	P_2	5.33 ± 0.58	6.67 ± 0.58	6.33 ± 0.58	7.33 ± 0.56	7.33 ± 2.08	7.67 ± 1.53	8.33 ± 0.58
Natural products								
Cinnamon	P_1	4.33 ± 0.58	5.67 ± 0.58	5.67 ± 0.58	6.33 ± 0.58	6.67 ± 0.58	7.67 ± 0.58	8.33 ± 1.16
	P_2	4.33 ± 0.58	5.67 ± 0.58	5.67 ± 0.58	6.33 ± 0.58	6.67 ± 0.58	7.67 ± 0.58	8.33 ± 1.16
Clove	P_1	4.67 ± 1.16	5.33 ± 0.58	5.33 ± 0.58	6.33 ± 0.58	6.67 ± 1.16	7.33 ± 0.58	8.00 ± 1.00
	P_2	4.67 ± 1.16	5.33 ± 0.58	5.33 ± 0.58	6.33 ± 1.53	7.00 ± 1.00	7.33 ± 1.16	8.00 ± 1.00
Garlic	P_1	4.00 ± 0.00	5.33 ± 0.58	5.33 ± 0.58	6.00 ± 1.00	6.00 ± 1.00	6.67 ± 1.53	7.00 ± 2.00
	P_2	4.00 ± 0.00	5.33 ± 0.58	5.33 ± 0.58	6.33 ± 0.58	6.00 ± 1.00	6.67 ± 1.16	7.00 ± 1.73
Mustard	P_1	5.33 ± 0.58	6.67 ± 0.58	6.33 ± 0.58	7.00 ± 1.00	7.00 ± 1.00	7.67 ± 1.53	8.33 ± 0.58
	P_2	5.33 ± 0.58	6.67 ± 0.58	6.33 ± 0.58	7.33 ± 0.58	7.33 ± 2.03	7.67 ± 1.53	8.33 ± 0.58
Turmeric	P_1	4.67 ± 1.16	5.33 ± 0.58	6.33 ± 0.58	6.67 ± 0.58	7.00 ± 1.00	7.33 ± 0.58	8.33 ± 0.58
	P_2	4.67 ± 1.16	5.33 ± 0.58	6.33 ± 0.58	6.67 ± 0.58	7.00 ± 1.00	7.33 ± 0.58	8.33 ± 0.58
Synthetic preservatives								
Acetic acid	P_1	5.00 ± 1.00	7.33 ± 0.58	8.33 ± 0.58	8.67 ± 0.58	8.67 ± 0.58	7.67 ± 1.16	6.33 ± 0.58
	P_2	5.00 ± 1.00	7.33 ± 0.58	8.33 ± 1.16	8.67 ± 0.00	8.67 ± 0.58	7.67 ± 1.53	6.67 ± 1.16
Potassium Sorbate	P_1	4.33 ± 0.56	5.67 ± 0.58	5.67 ± 0.58	6.33 ± 0.58	6.33 ± 0.58	7.00 ± 1.00	7.33 ± 1.53
	P_2	4.33 ± 0.56	5.67 ± 0.58	5.67 ± 0.58	6.33 ± 1.53	6.33 ± 1.53	7.00 ± 1.73	7.33 ± 2.08
Sodium Nitrite	P_1	4.67 ± 1.16	6.33 ± 1.16	6.33 ± 0.58	7.00 ± 0.00	7.00 ± 0.00	7.67 ± 1.16	8.00 ± 1.00
	P_2	4.67 ± 1.16	6.33 ± 1.16	6.33 ± 0.58	7.00 ± 1.00	7.67 ± 1.16	7.67 ± 1.16	8.00 ± 1.73

The data represent the mean ± SD of three replicates; ANOVA Table A.13 is given in annexure; P_1 =HDPE jar, P_2 =Glass jar
Treatment CD at 5% = P_1 (0.10) ; P_2 (0.16) ; Storage CD at 5% =P_1 (0.10) ; P_2 (0.12)

(Buckley et al.,1995) Reduction in hardness could be due to breakage of connective and myofibrillar proteins by the proteolytic enzymes during pickling and subsequent storage of pickles. In this reference, Das (2002) has also reported that treatment of meat chunk with acetic acid resulted in extreme softening of the product.

4.1.4.4. Taste

Taste along with odour and texture provides an overall sensation. Compounds responsible for taste of any food are generally non-volatile at room temperature and therefore, interact with taste receptors located in taste buds of the tongue. The four important basic taste perceptions are provided by: sour, sweet, bitter and salty compounds. The tastes of meat products depend on the curing ingredients viz. salt, sugar, nitrite, spices, etc. Some spices taste sweet, some taste bitter, some are pungent and some are aromatic.

Table 4.16 presents the taste scores of different meat pickles. The control samples, on the day 1, exhibited a taste score of 7.0. An equivalent taste score was observed in case of pickles treated with potassium sorbate, or sodium nitrite. The treatment with mustard resulted in higher taste score, as compared to control. However, other treatments in the decreasing order as garlic > clove > cinnamon > acetic acid > turmeric, resulted in poorer taste scores as compared to control. The effect of pickling treatment (medium) was highly significant ($P<0.05$) on day 1. The mustard treated samples were most liked followed by control, potassium sorbate and sodium nitrite treated pickles. Turmeric treated pickle was least liked in comparison to other treatments. The reason for varied response to taste of different pickles may be attributed to the intrinsic characteristics of each preservative used as a treatment. The pleasant pungent aroma of mustard was most favourable while the typical taste of turmeric was least appreciated.

Table4.16 Effect of treatments on taste score in buffalo meat pickles during storage of 120 days at ambient temperature.

Samples		Taste values as a function of storage time (days)						
		0	20	40	60	80	100	120
Control (untreated)	P_1	7.00 ± 1.00	7.67 ± 1.16	7.67 ± 0.58	8.33 ± 0.58	8.33 ± 0.58	8.67 ± 0.58	8.67 ± 0.58
	P_2	7.00 ± 1.00	7.67 ± 1.53	7.67 ± 1.16	8.00 ± 1.73	8.33 ± 0.58	8.67 ± 0.58	8.67 ± 0.58
Natural products								
Cinnamon	P_1	5.33 ± 0.58	6.00 ± 2.00	6.00 ± 2.00	6.00 ± 0.00	6.33 ± 0.58	6.33 ± 1.16	6.33 ± 1.53
	P_2	5.33 ± 0. 58	6.00 ± 2.00	6.00 ± 2.00	6.00 ± 0.00	6.33 ± 0.58	6.33 ± 1.16	6.33 ± 1.53
Clove	P_1	5.67 ± 0. 58	6.33 ± 1.16	6.67 ± 1.16	6.33 ± 0.58	6.67 ± 0.58	6.33 ± 1.16	6.67 ± 1.53
	P_2	5.67 ± 0. 58	6.33 ± 1.16	6.67 ± 1.16	6.33 ± 1.16	6.67 ± 0.58	6.33 ± 1.16	6.67 ± 1.53
Garlic	P_1	6.00 ± 1.00	6.00 ± 2.00	6.00 ± 1.73	6.67 ± 1.53	6.33 ± 1.58	6.33 ± 1.16	6.33 ± 1.16
	P_2	6.00 ± 1.00	6.00 ± 2.00	6.00 ± 1.73	6.67 ± 1.53	6.33 ± 1.58	6.33 ± 1.16	6.33 ± 1.16
Mustard	P_1	7.33 ± 1.53	7.67 ± 0.58	7.67 ± 0.58	7.67 ± 1.16	7.67 ± 1.53	8.67 ± 0.58	8.67 ± 0.58
	P_2	7.33 ± 1.53	7.67 ± 0.58	7.67 ± 0.58	7.67 ± 1.16	7.67 ± 1.53	8.67 ± 0.58	8.67 ± 0.58
Turmeric	P_1	5.00 ± 1.00	5.33 ± 0.58	5.33 ± 0.58	5.33 ± 0.58	5.33 ± 0.58	5.33 ± 0.58	5.33 ± 0.58
	P_2	5.00 ± 1.00	5.00 ± 2.00	5.33 ± 0.58	5.33 ± 0.58	5.33 ± 0.58	5.33 ± 0.58	5.33 ± 0.58
Synthetic preservatives								
Acetic acid	P_1	5.33 ± 0.58	4.67 ± 0.58	3.67 ± 1.53	3.33 ± 1.16	2.00 ± 1.00	1.33 ± 0.58	1.00 ± 0.00
	P_2	5.33 ± 0.58	4.67 ± 0.58	3.67 ± 1.53	3.33 ± 1.16	2.33 ± 0.58	1.33 ± 0.58	1.00 ± 0.00
Potassium Sorbate	P_1	7.00 ± 1.00	7.33 ± 1.16	7.67 ± 1.53	7.67 ± 0.58	7.67 ± 0.58	7.67 ± 1.53	8.33 ± 0.58
	P_2	7.00 ± 1.00	7.33 ± 1.16	7.67 ± 1.53	7.67 ± 0.58	7.67 ± 0.58	7.67 ± 1.53	8.33 ± 0.58
Sodium Nitrite	P_1	7.00 ± 1.00	7.33 ± 1.16	7.67 ± 1.53	8.00 ± 0.00	8.00 ± 1.73	8.00 ± 1.73	8.33 ± 0.58
	P_2	7.00 ± 1.00	7.33 ± 1.16	7.67 ± 1.53	8.33 ± 0.58	8.33 ± 1.16	8.00 ± 1.73	8.33 ± 0.58

The data represent the mean ± SD of three replicates; ANOVA Table A.14 is given in annexure; P_1 =HDPE jar, P_2 =Glass jar
Treatment CD at 5% = P_1 (0.16) ; P_2 (0.18) ; Storage CD at 5% =P_1 (0.13) ; P_2 (0.14)

Acetic acid treatment, significantly ($P<0.05$) improved the taste of pickles with storage time. At the end of 120 days period of ambient storage, the mustard treated pickles was good as control and the taste scores of these two pickles improved by 1.34 and 1.67 units. (on hedonic scale), respectively. Nitrite treated pickle was also appreciated as nitrites retarded development of off-odours and off-colours during storage besides inhibiting the development of WOF and preserving the flavour of spice. In most cases, the packaging material did not have any significant effect on taste score. Only acetic acid treatment significantly ($P<0.05$) reduced the taste score of pickles during 120 days of storage.

Keeping in view the over all taste scores of pickles on day 1 and after completion of 120 days of storage, all the pickles, excluding only acetic acid treated pickles, scored the ranking between 'neither liked nor disliked' to 'liked very much'. This finding is contrary to the general feeling that there is a gradual loss in flavour during storage of meat (Howard and Lawrie, 1956) which arise due to the slow loss of highly volatile substances. It seems that salts, and spices used in this study prevents the loss of volatile compounds and maintain the flavour for long time. This is supported by the earlier observation that the flavour of cured meat is different from that of uncured commodity (Lawrie, 1991).

4.1.4.5. Palatability

Various food additives viz, spices, acids and salts, besides, onions, tomato, etc and the cooking usually improve the palatability of meat products. In this reference Table 4.17 presents the palatability scores of different types of meat pickles. The control samples, on the day 1, had palatability score of 7 (liked moderately). The similar palatability scores was noticed in case of mustard or sodium nitrite, treated pickles. Treatments with other spices, salts and acetic acid, in comparison to control, reduced the palatability of pickles significantly ($P<0.05$) in decreasing order; as

Table 4.17 Effect of treatments on palatability score in buffalo meat pickles during storage of 120 days at ambient temperature.

Samples		Palatability values as a function of storage time (days)						
		0	20	40	60	80	100	120
Control (untreated)	P_1	7.00 ± 0.00	7.00 ± 0.00	7.67 ± 0.58	8.00 ± 1.00	8.00 ± 1.00	8.33 ± 1.16	7.67 ± 1.53
	P_2	7.00 ± 0.00	7.00 ± 0.00	7.67 ± 0.58	8.00 ± 1.00	8.00 ± 1.00	8.33 ± 1.16	7.67 ± 1.53
Natural products								
Cinnamon	P_1	6.67 ± 0.58	7.67 ± 0.58	8.00 ± 1.00	7.67 ± 0.58	8.00 ± 1.00	8.33 ± 0.58	8.33 ± 0.58
	P_2	6.67 ± 0.58	7.67 ± 1.16	7.67 ± 1.16	7.67 ± 0.58	8.33 ± 0.58	8.67 ± 0.58	8.67 ± 0.58
Clove	P_1	6.33 ± 0.58	6.00 ± 0.00	6.33 ± 1.53	6.33 ± 0.58	6.33 ± 2.31	6.33 ± 1.53	6.33 ± 0.58
	P_2	6.33 ± 0.58	6.33 ± 1.53	6.33 ± 1.53	6.33 ± 0.58	6.33 ± 2.31	6.33 ± 0.58	6.33 ± 0.58
Garlic	P_1	6.33 ± 0.58	7.67 ± 1.53	7.67 ± 0.58	7.67 ± 1.16	8.00 ± 1.00	8.00 ± 1.00	8.00 ± 1.00
	P_2	7.00 ± 0.00	7.67 ± 0.58	8.00 ± 1.00	8.00 ± 1.00	8.00 ± 1.73	8.00 ± 1.00	8.00 ± 1.73
Mustard	P_1	7.00 ± 0.00	7.67 ± 0.58	8.00 ± 1.00	8.00 ± 1.00	8.33 ± 1.16	8.33 ± 0.58	8.33 ± 0.58
	P_2	7.00 ± 0.00	7.67 ± 0.58	8.00 ± 1.00	8.33 ± 0.58	8.33 ± 1.16	8.33 ± 0.58	8.33 ± 1.16
Turmeric	P_1	5.67 ± 1.16	5.67 ± 0.58	5.67 ± 2.08	5.67 ± 1.16	5.67 ± 1.53	5.67 ± 0.58	5.67 ± 1.53
	P_2	5.67 ± 1.16	5.67 ± 0.58	5.67 ± 2.08	5.67 ± 2.09	5.67 ± 2.09	5.67 ± 0.58	5.67 ± 2.08
Synthetic preservatives								
Acetic acid	P_1	5.67 ± 0.58	5.33 ± 0.58	5.33 ± 0.58	4.67 ± 0.58	4.00 ± 1.00	4.00 ± 1.00	2.67 ± 0.58
	P_2	5.67 ± 0.58	5.33 ± 0.58	5.33 ± 0.58	4.67 ± 0.58	4.67 ± 0.58	4.33 ± 1.53	2.67 ± 1.16
Potassium Sorbate	P_1	6.33 ± 0.58	7.33 ± 1.53	7.33 ± 0.58	7.33 ± 1.53	7.67 ± 1.53	8.33 ± 0.58	8.33 ± 0.58
	P_2	6.33 ± 0.58	7.33 ± 1.53	7.33 ± 0.58	7.33 ± 1.53	7.67 ± 1.53	8.33 ± 0.58	8.33 ± 0.58
Sodium Nitrite	P_1	7.00 ± 1.73	7.33 ± 1.16	7.67 ± 1.53	7.00 ± 2.00	7.67 ± 1.53	8.00 ± 1.00	8.00 ± 1.00
	P_2	7.00 ± 1.73	7.67 ± 1.53	8.00 ± 1.00	7.67 ± 1.16	8.33 ± 0.58	8.33 ± 1.16	8.33 ± 0.58

The data represent the mean ± SD of three replicates; ANOVA Table A.15 is given in annexure; P_1 =HDPE jar, P_2 =Glass jar
Treatment CD at 5% = P_1 (0.15) ; P_2 (0.16) ; Storage CD at 5% =P_1 (0.12) ; P_2 (0.13)

cinnamon > clove, or garlic, or potassium sorbate > turmeric or acetic acid. The intrinsic flavour of these additives in pickling mediums may be the reason of such varied palatability scores. While the control and mustard treated pickles were ranked as 'liked moderately', the turmeric and acetic acid treated pickles on day 1 were ranked as 'neither liked nor disliked'.

Various pickling treatments had ($P<0.05$) significant effect on palatability scores of pickles which were stored for 120 days at ambient temperatures. The palatability scores of control samples, cinnamon, garlic, mustard, potassium sorbate and sodium nitrite treated pickles improved significantly ($P<0.05$) during 120 days storage while in case of the acetic acid treated pickles, the palatability score decreased to a significant ($P<0.05$) extent. However, treatments with clove and turmeric did not change the palatability score of pickles during 120 days storage.

In case of acetic acid the palatability score decreased by three units on 9 point hedonic scale, however, the score increased by two units in case of treatments with potassium sorbate followed by garlic and cinnamon treatment (1.67 units), mustard (1.33 units), sodium nitrite (1 unit) and control (0.67 units). At the end of 120 days storage, the cinnamon, mustard, potassium sorbate and sodium nitrite treated pickles packed in glass jar were most liked. Except in case of treatments with cinnamon and sodium nitrite, there was no significant effect of packaging materials on palatability score. In these two treatments glass jar proved better than HDPE jar because of better barrier properties.

Decline in pH of pickles during storage (Table 4.1) as a result of fermentation, which provided tangy flavour and chewy texture, may be the reason for increase in palatability of pickles.

4.1.5.Textural profile analysis (TPA)

Texture is a function of the size of the bundles of fibers into which the perimysial septa of connective tissue divide the muscle longitudinally (Hammond, 1932). There is an indirect correlation between muscle fibre diameter and tenderness (Hiner et al., 1953). This emphasize the complexity of texture and tenderness as attributes of eating quality. The overall impression of tenderness to the palate includes texture, and involves three aspects i.e. (i) the initial ease of penetration of meat by the teeth, (ii) the ease with which the meat breaks into fragments and (iii) the amount of residue remaining after chewing (Weir, 1960)

In light of above, the data pertaining to hardness and cohesiveness (experimental values) and gumminess (calculated values) are presented in Table 4.18.

4.1.5.1. Hardness

Based on the behaviour of food in mouth, hardness is defined as force necessary to attain a given deformation. It is regarded as an important textural parameter related to maturity, ripeness, storability and tenderness. In the plots of force vs. time (Figs 4.3 to 4.11) showing the textural profile of a meat pickles, the positive peak force of first bite (compression cycle) represents the hardness of the product. The ratio of the positive force area (PA_2) during the first compression cycle to the PA_2 / PA_1 provides the information related to cohesiveness of the meat pickles. Table 4.18 shows the hardness values of different types of meat pickles developed in the present study.

It may be noted that the hardness value of control sample of meat pickle on day1 was 2.21 $x10^4$ g . In comparison to this value the corresponding value of hardness of different types of preservative treated pickles varied between 2.0 $x10^4$ g to 2.21 $x10^4$ g showing that the various preservatives marginally affected the hardness of pickle on day 1. Turmeric, mustard, garlic and cinnamon treated pickles had lower

Table 4.18 Textural analysis of different types of meat pickles.

Samples		Hardness (g)	Cohesiveness		Gumminess (g)
		P^1	PA_2	$PA_2\backslash PA_1$	$P^1 x PA_2\backslash PA_1$
Control (untreated)	Day 1	2.21×10^4	5.49×10^4	1.38	3.05
	P_1	0.71×10^4	2.50×10^4	2.08	1.48
	P_2	0.78×10^4	2.55×10^4	0.99	0.77
Natural products					
Cinnamon	Day 1	2.07×10^4	5.49×10^4	1.38	2.85
	P_1	0.60×10^4	8.32×10^3	1.87	1.12
	P_2	0.58×10^4	8.31×10^3	1.87	1.08
Clove	Day 1	2.21×10^4	5.49×10^4	1.38	3.05
	P_1	0.78×10^3	3.30×10^3	0.47	3.67
	P_2	0.79×10^3	3.31×10^3	0.47	3.72
Turmeric	Day 1	2.00×10^4	5.49×10^4	1.38	2.76
	P_1	2.25×10^4	8.64×10^4	1.04	2.34
	P_2	2.30×10^4	6.63×10^4	1.16	2.66
Garlic	Day 1	2.07×10^4	5.49×10^4	1.38	2.86
	P_1	0.27×10^4	1.12×10^4	2.55	0.69
	P_2	0.41×10^4	9.67×10^4	2.44	1.00
Mustard	Day 1	2.02×10^4	5.49×10^4	1.38	2.79
	P_1	0.40×10^4	2.24×10^4	3.20	1.28
	P_2	0.49×10^4	2.69×10^4	3.85	1.89
Synthetic preservatives					
Potassium sorbate	Day 1	2.21×10^4	5.49×10^4	1.38	3.07
	P_1	2.25×10^4	7.11×10^4	1.95	4.38
	P_2	2.35×10^4	7.11×10^4	1.95	4.58
Sodium nitrite	Day 1	2.21×10^4	5.49×10^4	1.38	3.05
	P_1	0.41×10^4	2.24×10^4	3.19	1.31
	P_2	0.95×10^4	1.31×10^4	1.61	1.53
Acetic acid	Day 1	2.21×10^4	5.49×10^4	1.38	3.05
	P_1	0.06×10^2	2.72×10^3	1.11	0.67
	P_2	0.06×10^2	2.72×10^3	1.11	0.68

Day 1:- Textural data of fresh pickles on day1, P_1 :- Data after 120 days of storage in HDPE jar, P_2 :-Data after 120 days of storage in glass jar, P^1 :- positive peak force, PA_1 :- area of first; PA_2 :- area of second bite,

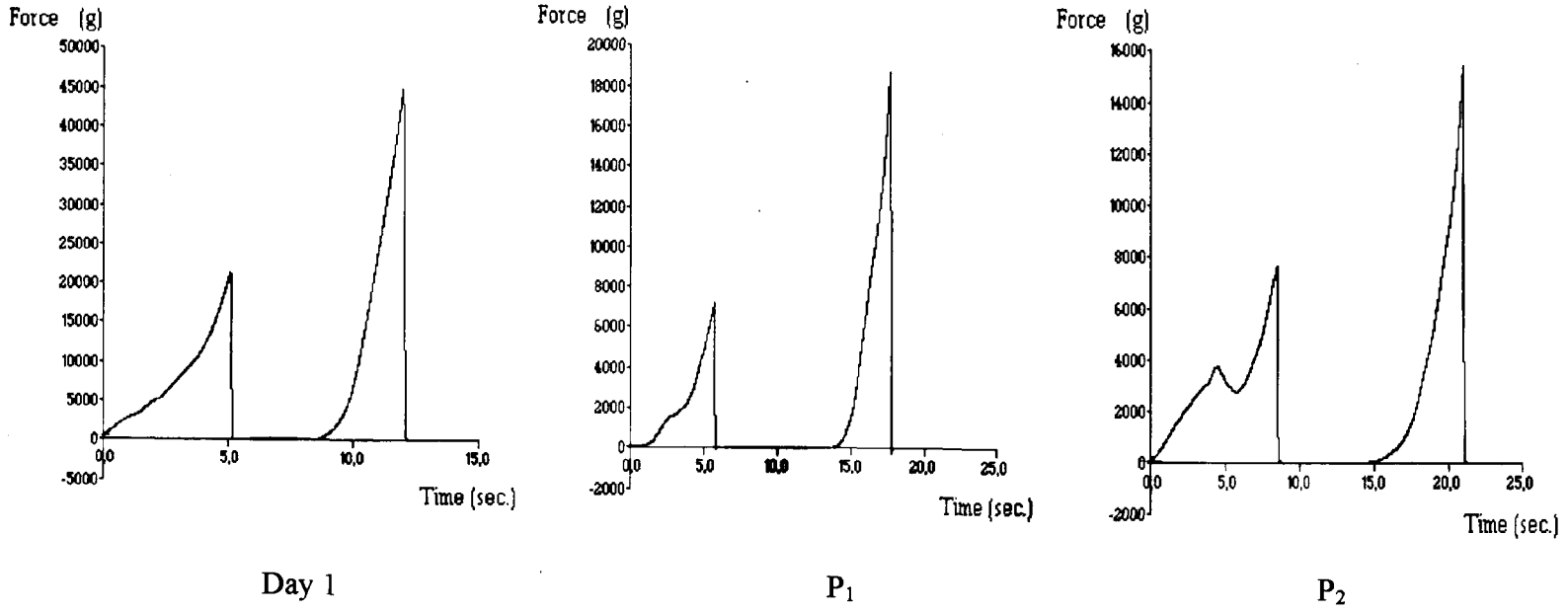


Fig 4.3. Textural characteristics of control meat pickle on Day 1 and after 120 days preservation at ambient temperature for 120 days at ambient temperature.

Day 1:- Textural characteristics of fresh pickles. P_1 and P_2 graphs represent the textural characteristics of pickles after 120 days storage in HDPE and glass jars, respectively.

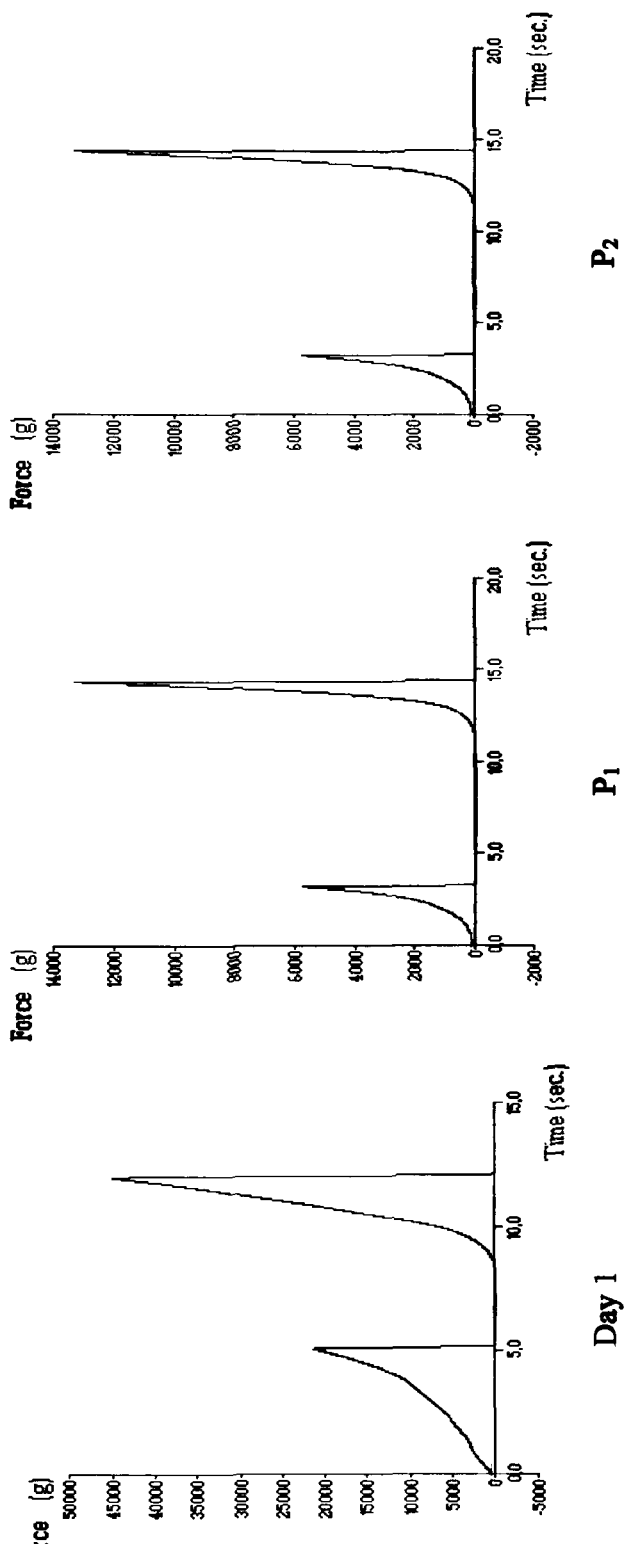


Fig 4.4. Force vs. time plot represents the textural **characteristics of cinnamon meat pickle on Day 1 and after 120 days** preservation at ambient temperature for

Day 1:- Textural characteristics of fresh pickles. P_1 and P_2 graphs represent the textural characteristics of pickles after 120 days storage in HDPE and glass jars, respectively.

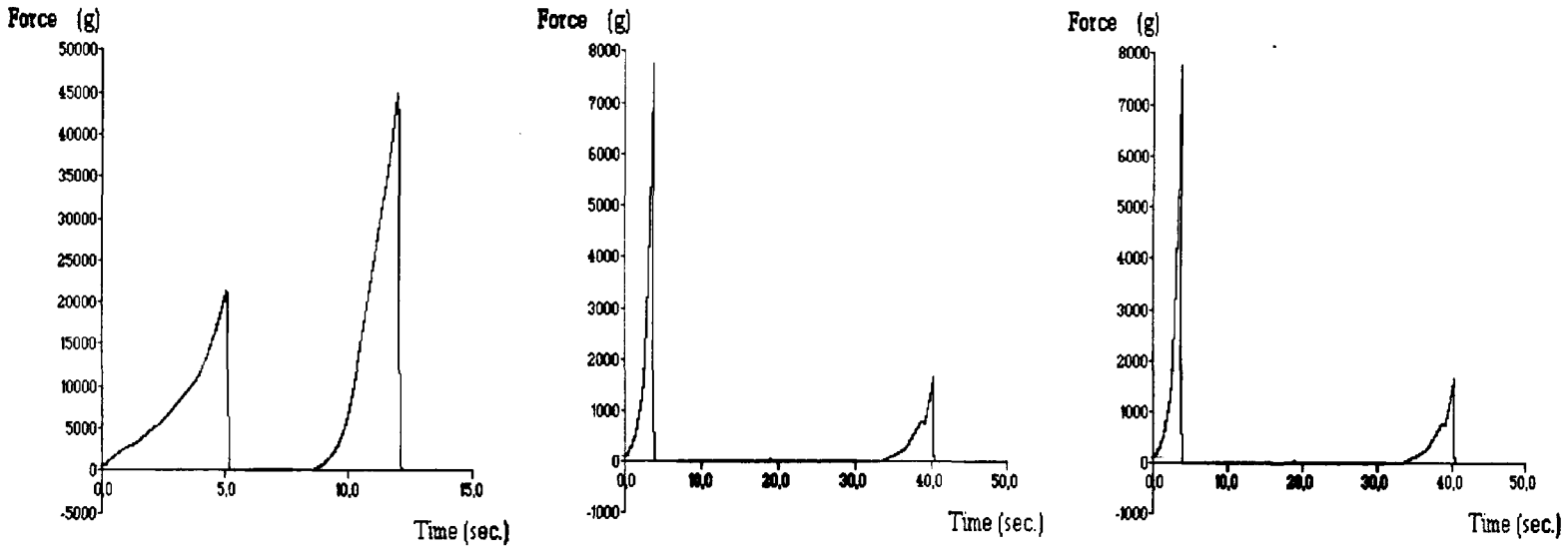


Fig 4.5 Textural characteristics of clove treated pickle on Day 1 and after 120 days preservation at ambient temperature for 120 days at ambient temperature.

Day 1 :-Textural characteristics of fresh pickles. P_1 and P_2 graphs represent the textural characteristics of pickles after 120 days storage in HDPE and glass jars, respectively.

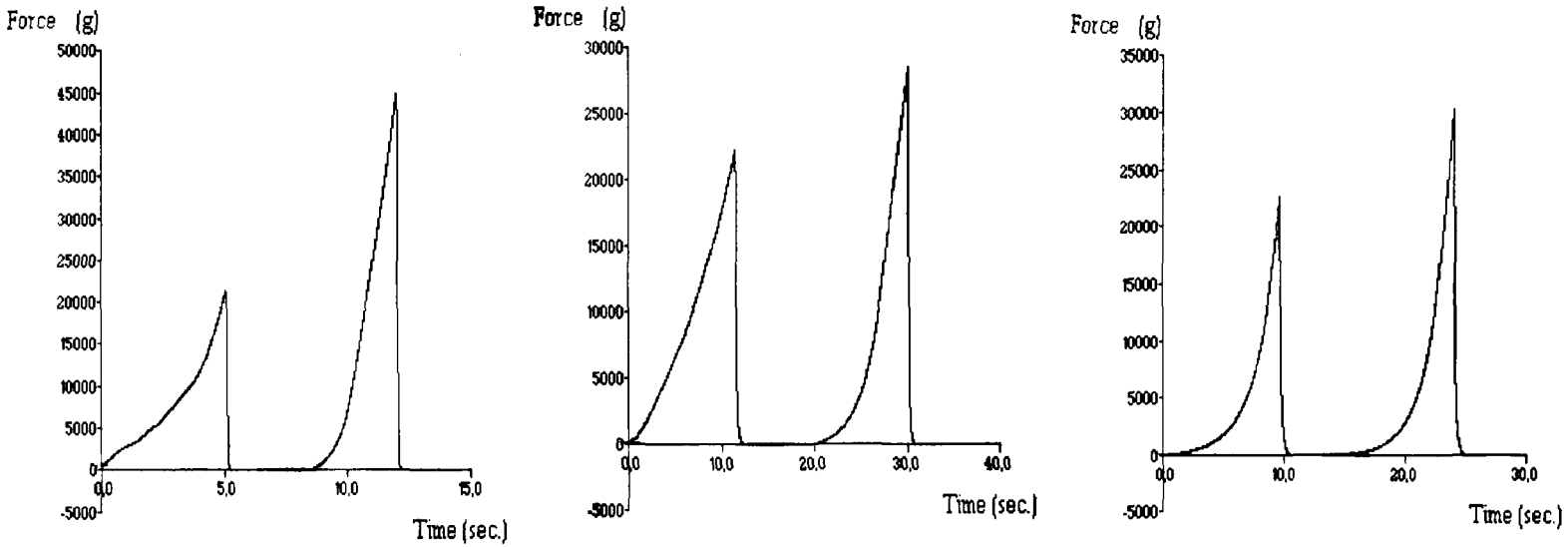


Fig 4.6. Force vs. time plot represents the textural characteristics of turmeric treated buffalo meat pickle on Day 1 and after 120 days preservation at ambient temperature for

Day 1 :- Textural characteristics of fresh pickles. P_1 and P_2 graphs represent the textural characteristics of pickles after 120 days storage in HDPE and glass jars, respectively.

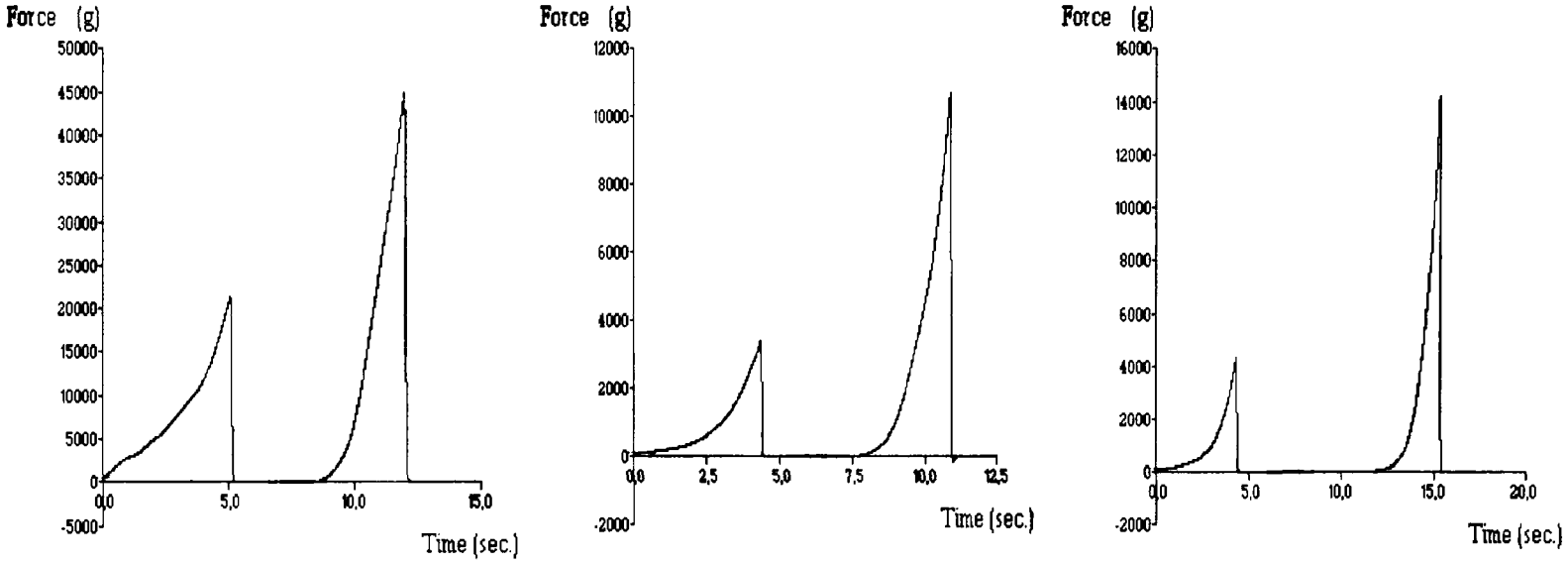


Fig 4.7. Force vs time plot represents the textural characteristics of garlic **treated** buffalo **meat** pickle on Day 1 **and** after 120 days preservation at ambient temperature for

Day 1:- Textural characteristics of fresh pickles. P_1 and P_2 graphs **represent** the **textural** characteristics **of** pickles after 120 days storage in HDPE and glass jars, respectively.

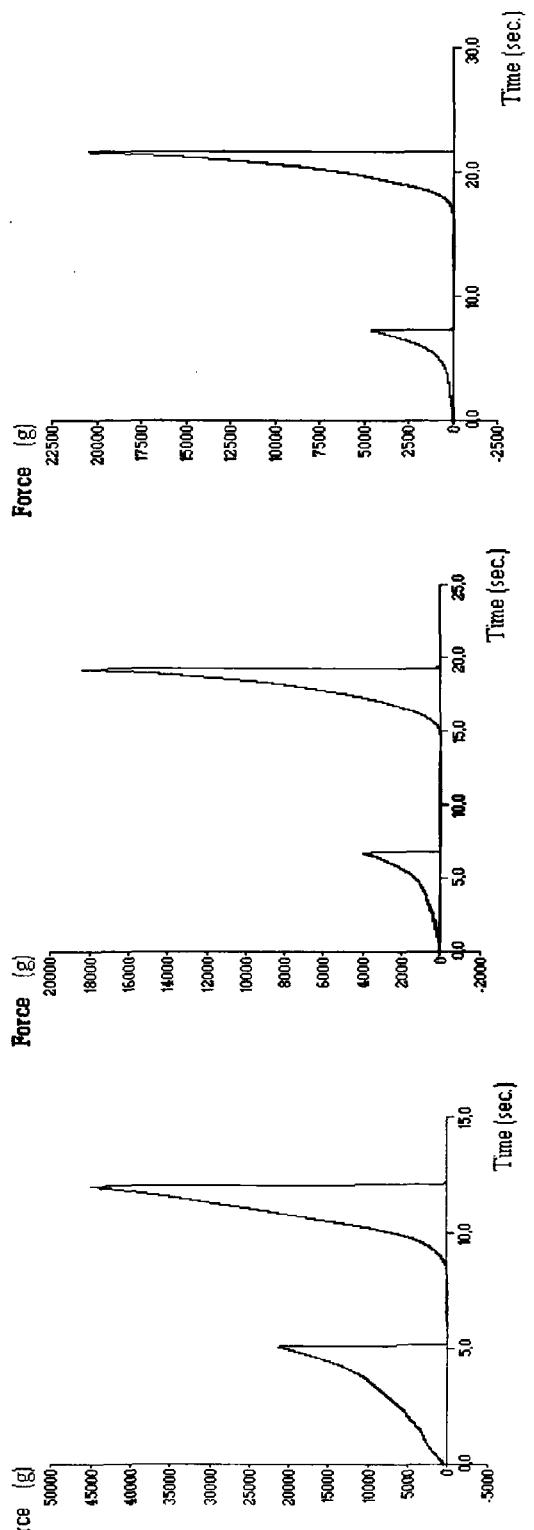
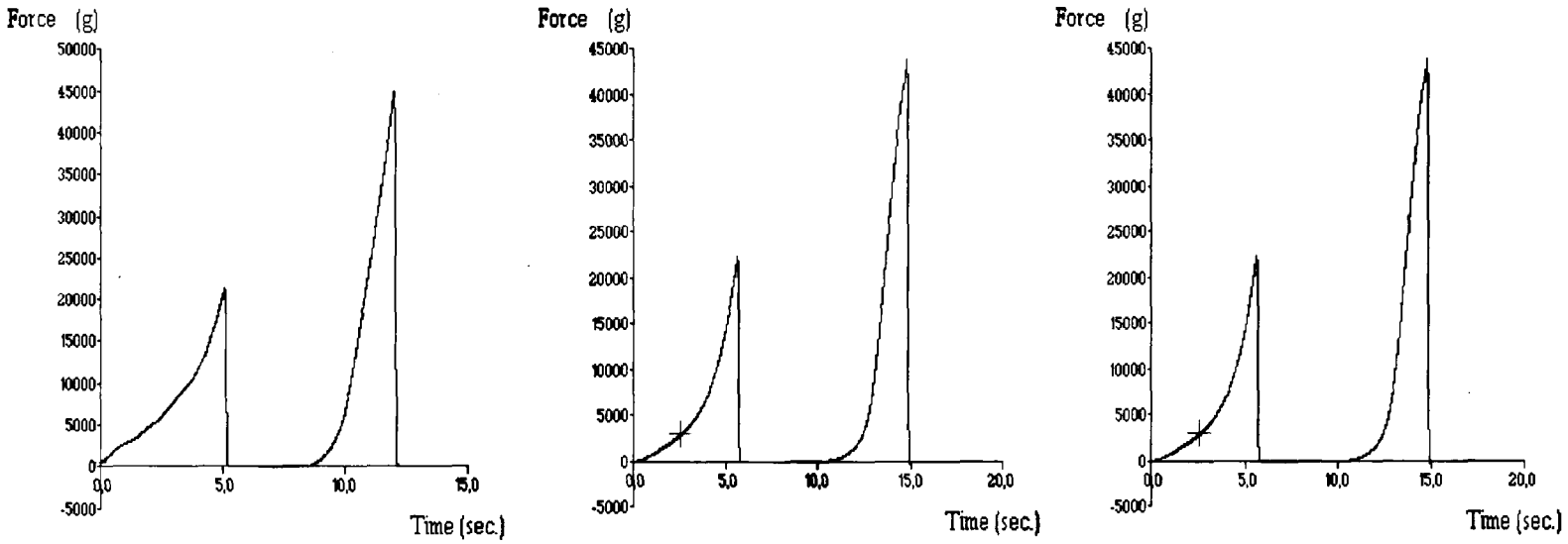


Fig 4.8 Force vs. time plot represents the textural characteristics of mustard treated buffalo meat pickle on Day 1 and after 120 days preservation at ambient temperature for

Day 1:- Textural characteristics of fresh pickles. P_1 and P_2 graphs represent the textural characteristics of pickles after 120 days storage in HDPE and glass jars, respectively



Day 1 P_1 P_2

Fig 4.9. Force vs. time plot represents the textural characteristics of **potassium sorbate treated buffalo** meat **pickle on Day 1** and after 120 days preservation at ambient temperature for

Day 1:- Textural characteristics of fresh pickles. P_1 and P_2 **graphs represent the textural characteristics of pickles** after 120 days storage in HDPE and glass jars, respectively

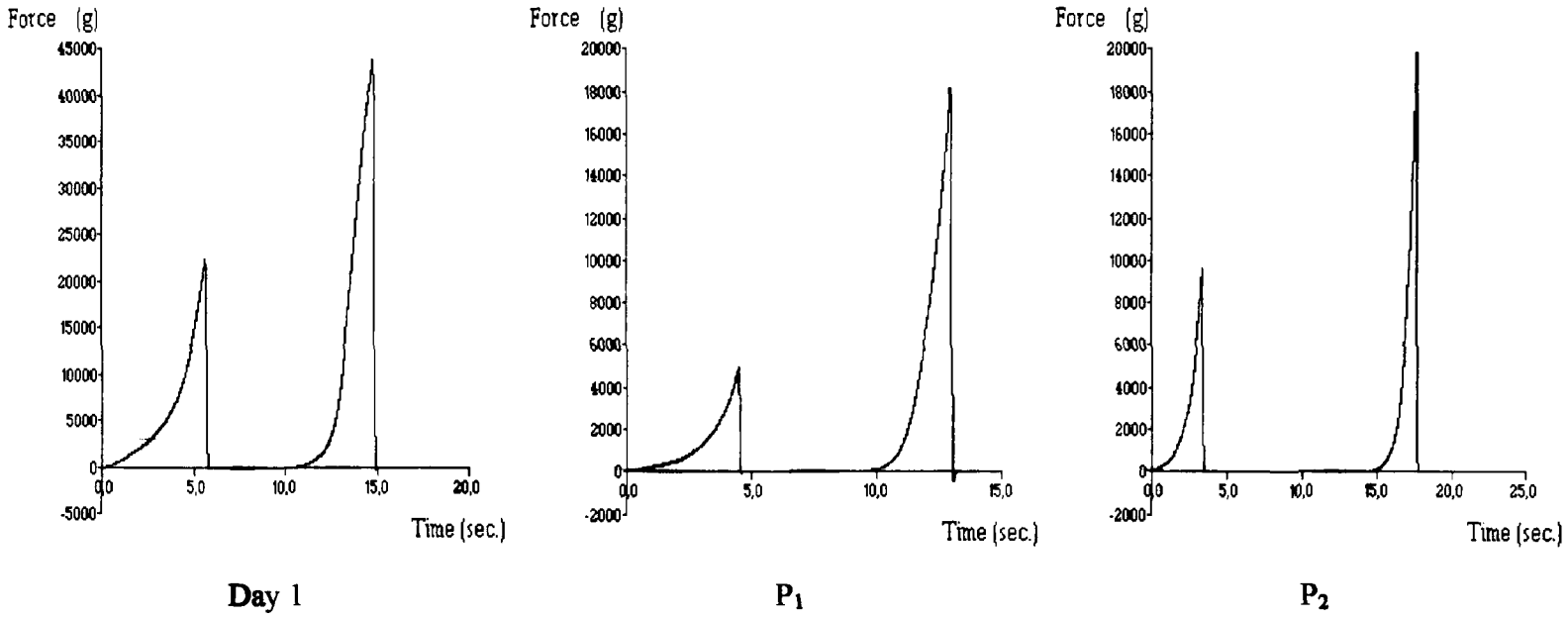


Fig 4.10 Force vs. time plot represents the textural characteristics of sodium nitrite treated buffalo meat pickle on Day 1 and after 120 days preservation at ambient temperature for

Day 1:- Textural characteristics of fresh pickles. P_1 and P_2 graphs represent the textural characteristics of pickles after 120 days storage in HDPE and glass jars, respectively

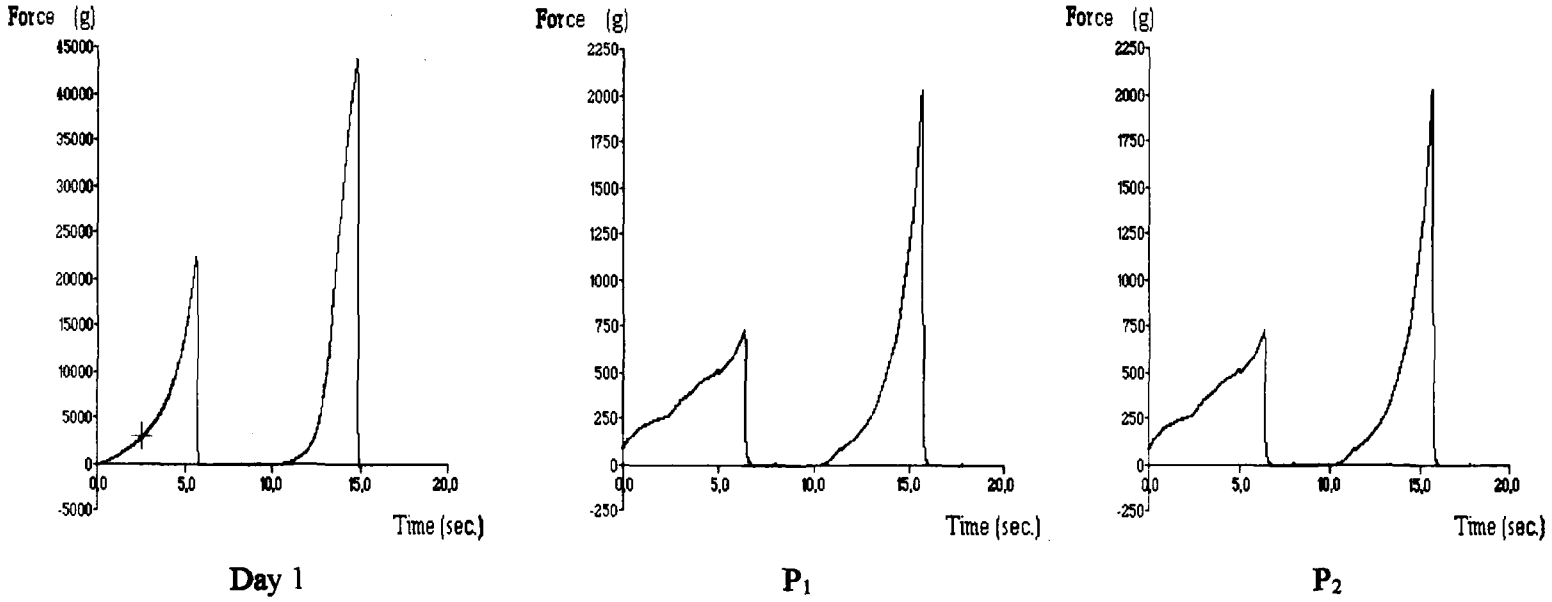


Fig 4.11. Force vs. time plot represents the textural characteristics of **acetic acid treated buffalo meat pickle on Day** 1 and after 120 days preservation at ambient temperature for

Day 1 :- Textural characteristics of fresh pickles. P_1 and P_2 graphs represent the textural characteristics of pickles after 120 days storage in HDPE and glass jars, respectively

values of hardness as compared to cloves, potassium sorbate, sodium nitrite and acetic acid treated meat pickles. The reduction in hardness is attributed to breakage of connective and mycofibrillar proteins due to proteolytic enzymes during pickling operation.

Reduction in hardness of pickles contributes to improvement of tenderness of pickles. It seems that the constituents of spices such as cinnamon, turmeric, garlic, and mustard might have facilitated meat tenderization in pickles as compared to clove and synthetic preservatives. During ambient storage of pickles for 120 days in HDPE and glass jars varied response in hardness values were observed.

In general, the hardness of pickles decreased in most of the cases except the treatments with turmeric and potassium sorbate. Maximum reduction in hardness was observed in acetic acid treated pickle followed by garlic/sodium nitrite, mustard, cinnamon and clove treated pickles. The varied tenderizing effects of different preservatives/ additives spices, salts/acetic acid used in the present investigation may be understood in the light of desirable proteolysis of muscle fibre proteins. It has been reported that the digestion of sarcolemma cause disappearance of nuclei followed by degradation of muscle, fibre, eventually resulting in loss of cross-striations. Also, the connective tissue fibres are degraded, due to proteolysis action on mucopolysaccharides of ground substances matrix to an amorphous mass. Moreover, the connective tissue proteins on degradation yield soluble hydroxy- proline containing molecules. Sodium chloride and other salts also exert weak tenderizing action on meat (Wang et al., 1958). The results of hardness of pickles are in accordance with studies of Pawar et al. (2002) who reported that hardness of meat patties decreased with addition of fat but increased on addition of whey protein concentrate. Khan (2004) has also reported decrease in hardness of buffalo meat due to curing and treatment with sodium ascorbate. According to Khan (2004), the

hardness of kabab decreased with increase in storage period depending upon the method of packaging used.

Pickles packed in HDPE jar had slightly lower hardness values as compared to glass jar packed pickles, probably due to poorer water vapour barrier properties of HDPE as compared to glass which resulted in softening of packed pickles.

4.1.5.2.Cohesiveness

Cohesiveness of a food material determines the extent of deformation the material can with stand before it ruptures. In the plot of force vs time (Figs 4.3 to 4.11), it is obtained as a ratio of area of second bite to area of first bite. Being a ratio, it is dimensionless.

The control meat pickles, on day 1, had cohesiveness value of 1.38 as was the case with cinnamon, turmeric, garlic, mustard, potassium sorbate and sodium nitrite treated meat pickles. In comparison to control samples, the pickles treated with clove (cohesiveness value, 2.45) and acetic acid (cohesiveness value, 2.08) had higher cohesiveness. The reasons for varied cohesiveness of different types of meat pickles may be due to (i) the extent of changes in fat and protein contents, (ii) action of proteolytic and lipolytic enzymes, and (iv) consequent protein denaturation as earlier discussed. Among the various treatments, clove and acetic acid treatments resulted in maximum disintegration of muscle fibres and accordingly pickles treated with these preservatives had higher cohesiveness as compared to other treatments.

The cohesiveness of meat pickles during 120 days storage in HDPE jar increased in case of control, garlic, mustard, potassium sorbate and sodium nitrite treatments. Maximum increase in cohesiveness of pickles packed in HDPE jar during storage was observed in case of sodium nitrite treatment (increase by 1.81) and mustard treatments (increase by 1.82) followed by garlic treatment (increase by 1.17). In case of some pickles packed in glass jar, there was however, considerable decrease

in cohesiveness of control pickles during storage (decrease 0.61) as against increase in corresponding value during storage in HDPE jars.

Maximum decrease in cohesiveness during storage was observed in case of clove (decreased by 1.98) followed by acetic acid treatment. This showed that storage period and treatment, both have considerable effects on cohesiveness of pickles. However, among various treatments, packaging materials had effect only in case of control samples and sodium nitrite treated samples. Typical changes in fat content of pickles during storage may be attributed for such varied response of storage period and packaging materials on cohesiveness. Pawar et al. (2001) have also reported that the cohesiveness of chevon patties showed a definite increase by addition of fat and decrease owing to the addition of whey protein concentrate at different levels. However, Berry and Leddy, (1984) did not find any significant effect of addition of fat on the cohesiveness of ground beef patties when tested by sensory methods. Keeton (1983) also did not report any significant change in cohesiveness of pork patties with increasing levels of fat from 20 to 30%. Khan (2004) had reported that curing did not change the cohesiveness of buffalo meat Kabab, however, it changes with the change in packaging systems, storage period and antioxidants used.

4.1.5.4 Gumminess

Gumminess of semi-solid foods is the force required to disintegrate it to a state ready for swallowing. It is affected by hardness and cohesiveness of the product. The control samples on day 1 had gumminess of 3.05×10^4g. In comparison to control the acetic acid (4.1×10^4 g), and clove (8.27×10^4 g) treated samples had higher gumminess while cinnamon (2.85×10^4g), turmeric (2.76×10^4 g), garlic (2.86×10^4g) and mustard (2.79×10^4g), treated pickles had lower gumminess values. Also, considerable changes in gumminess of potassium sorbate and sodium nitrite treated pickles were noticed in comparison to control samples. These changes were due to

varying extent of protein denaturation and changes in fat content due to different pickling treatments, which have also significantly affected the hardness and cohesiveness of pickles.

Maximum reduction in gumminess of acetic acid treated pickles (by 0.67 and 0.68 g) was noted during 120 days storage in HDPE and glass jars, respectively, as the treatment resulted in maximum disintegration of muscle fibres. This suggest that the packaging material in specific combination of spices and chemical preservatives influences the gumminess of pickles.

4.1.6 Shelf life of hurdle processed meat pickles

Meat is a highly perishable food product with very short shelf life due to its high moisture and protein contents, which are utilized by microorganisms for their growth. The spoilage of muscle tissue is the critical factor determining the shelf life of meat and its products. An unfavorable environment for growth of microorganisms can extend the shelf life of meat and its products. Various hurdle parameters viz. water activity, redox potential, preservatives, pH, packaging, etc. are used to check the growth of microorganisms, which in turn extend the shelf life of meat and meat products. Dehydration, salting or curing, use of organic acid and modified atmosphere storage are some of the measures commonly employed to retard meat spoilage.

In above reference, Ranken (2000) recommended that meat deboned under good condition may contain surface viable count of microorganisms to the extent of $1x10^4$ CFU/g or per cm^2, and meat with count of $1x10^6$ CFU/g should be rejected. Spoilage of meat is considered when the count of microorganisms reaches to approximately $1x10^6$ organisms CFU/g. According to Bauemann (1979), the level of log value of total plate counts upto 5 CFU/g is considered as the maximum limit for acceptability of the products and the values of 7 is indicative of starting of spoilage (Panda,1971). For meat and its products the shelf life is limited due to lipid oxidation,

which is influenced by intrinsic characteristics of meat, unsaturated fatty acids contents and presence of pro-oxidants and anti-oxidants. Anti-oxidants enhance the oxidation stability as well as the shelf life of meat products. Among organic acids, short chain length non fatty acids such as acetic acid are most effective. (Geopfert and Hicks, 969). However, these acids are biologically active only in the undisassociated form. The degree of dissociation increases with increasing pH so there is a very marked increase in biological activity with decreasing pH. Low pH of meat products result in greater product stability. In case of fermented meat products, fermentation takes place due to acid development, which inhibits undesirable microorganisms and destabilizes the protein with accompanying moisture loss. Low acid fermented meat products have a final pH of about 6.0 with preservation being achieved mainly by drying at low temperatures. High acid fermented products, on the other hand, have a final pH of 5.3 or lower, which is achieved by a combination of acid production by inoculation with bacteria (naturally present or as cultures) and drying. Lowering the water activity (reduction in moisture content) also is important in preventing meat spoilage. Too low moisture content of dehydrated meat does not permit bacterial growth. However, if the moisture content of dehydrated meat rises above 10%, mold growth may occur after some weeks (Lawrie, 1991).

Antibiotics might also be usefully employed to control spoilage bacteria, which are very heat resistant and can not other wise be killed unless the product is given a heat treatment so excessive that the texture of meat is damaged (Hansen and Riemann, 1958).

Vinegar (acetic acid) and herbs or spices have been traditionally used to steep fish and meat and for pickling though these do not come under category of preservatives. CO_2 is also a chemical used for modified atmosphere

packaging/storage, which discourages the growth of surface microorganisms on beef carcass, etc during prolonged storage at chill temperatures.

Among various spices used in present studies, mustard is anti-microbial while garlic is antibacterial, clove acts both as antioxidant as well as anti-microbial while turmeric is antioxidant. In light of these discussions, the shelf lives of different types of developed buffalo meat pickles were evaluated.

Keeping in view the limit of acceptability of foods with $1x10^6$CFU/g microorganisms or its log value of 5 as indicated above, all the samples of meat pickles during 120 days storage in HDPE and glass jars were found to be within safe limits. Thus, the developed buffalo meat pickles, irrespective of pickling treatments and packaging materials had a shelf life of 180 days at ambient temperature.

Product two: hurdle processed meat powder

4.2. Development and quality evaluation of buffalo meat powder

In addition to curing and pickling, the drying of meat is another effective method of checking meat spoilage. Drying / dehydration occurs as a result of moisture removal from meat. It is one of the known methods of dry curing in which meat is packed in salt. As the salt penetrates into the meat, the water activity within the meat is reduced. Smoking also results in dehydration of meat and is an other well-known method of preventing the spoilage of meat.

In view of the historical background and technical feasibility of dehydration as an effective method to check meat spoilage, efforts have been made in present investigation to use certain hurdle parameters for developing meat powder (Fig 4.12). For this purpose, two natural products viz. (cloves and turmeric) and potassium sorbate, as a chemical preservative were chosen based on their antimicrobial and antifungal properties. Modified atmosphere packaging and vacuum packaging were also employed as a hurdle factor.

Fig 4.12 Dehydrated buffalo meat powder in combination film and autoclaveable polythene packed by atmosphere, vacuum and MAP packaging methods.

Using these preservatives as specific treatments, the shelf life of packaged mat powder in combination film and autoclaveable polythene has been assessed. The storage studies were carried out under three different conditions. The data on the physico-chemical, microbiological and organoleptic characteristics of meat powder, in fresh condition and during 120 days preservation are discussed below:

4.2.1. Physico-chemical characteristics

4.2.1.1. Average particle size

Average particle size of meat powder is presented in Table 4.19. The values of xi (mass fraction retained) and Dpi (average particle diameter) were obtained and computed using the algorithm $D_s = 1 / \Sigma \frac{xi}{Dpi}$. The average particle size of meat powder was determined to be 0.26 mm.

4.2.1.2. Moisture content

Dehydration results in direct removal of moisture. Loss of water from both the raw and pre-cooked meat is accompanied by diminishing the space between the groups of muscle fibres and by progressive reduction in muscle fibre diameter (Wang et al.,1953). The rate of moisture removal and of muscle fibre shrinkage is more rapid with pre-cooked meat than with raw meat.

The moisture contents of meat powder as an indicator of water activity are presented in Table 4.20. The results show that the moisture content of treated meat powder vary in the range from 4.00 to 4.22 % as compared to 75 % moisture in raw meat.

The moisture contents of meat powders during storage have not changed significantly except the marginal decrease noticed under certain treatment conditions. Also, no effect at atmosphere packaging and packaging material was observed. Since no published information on meat powder is available, it is difficult to make the

Table 4.19 The average particle size of meat powder

S.no	Mesh No	x_i	Dpi(mm)	xi / Dpi
1	028 / 030	0.224	0.545	0.411
2	030 / 052	0.272	0.398	0.684
3	052 / 060	0.176	0.273	0.645
4	060 / 100	0.203	0.201	1.012
5	100 / 150	0.099	0.127	0.78
6	150 / 170	0.002	0.097	0.024
7	170 / 200	0.024	0.081	0.291
				∑3.85

xi = Mass fraction retained

Dpi = Average particles diameter in crements

D_s =Volume surface mean diameter

Table 4.20 Effect of treatments on moisture content of buffalo meat powder during storage of 120 days at ambient temperature.

Samples		Moisture content (%) as a function of storage time (days)						
		0	20	40	60	80	100	120
Control (Untreated)	P_3	4.22 ± 0.300	4.22 ± 0.321	4.21 ± 0.321	4.21 ± 0.300	4.22 ± 0.321	4.22 ± 0.381	4.33 ± 0.350
	P_4	4.22 ± 0.300	4.23 ± 0.755	4.20 ± 0.353	4.20 ± 0.300	4.21 ± 0.780	4.31 ± 0.400	4.31 ± 0.321
Natural products/Preservative								
Clove	P_3	4.00 ± 0.061	4.00 ± 0.100	4.00 ± 0.057	4.00 ± 0.200	4.00 ± 0.152	4.00 ± 0.063	4.00 ± 0.000
	P_4	4.00 ± 0.061	4.00 ± 0.061	4.00 ± 0.155	4.00 ± 0.060	4.00 ± 0.060	4.00 ± 0.063	4.00 ± 0.063
Turmeric	P_3	4.12 ± 0.155	4.10 ± 0.293	4.11 ± 0.380	4.13 ± 0.461	4.10 ± 0.152	4.10 ± 0.063	4.10 ± 0.100
	P_4	4.12 ± 0.155	4.10 ± 0.293	4.13 ± 0.382	4.11 ± 0.672	4.10 ± 0.100	4.10 ± 0.230	4.10 ± 0.061
Potassium sorbate	P_3	4.10 ± 0.121	4.10 ± 0.315	4.10 ± 0.312	4.10 ± 0.263	4.10 ± 0.231	4.00 ± 0.212	4.00 ± 0.212
	P_4	4.10 ± 0.177	4.10 ± 0.351	4.10 ± 0.577	4.10 ± 0.263	4.10 ± 0.231	4.00 ± 0.212	4.00 ± 0.212
Packaging methods								
CO_2 flushing (MAP)	P_3	4.10 ± 0.100	4.10 ± 0.100	4.10 ± 0.100	4.10 ± 0.100	4.10 ± 0.100	4.10 ± 0.100	4.10 ± 0.100
	P_4	4.10 ± 0.100	4.10 ± 0.100	4.10 ± 0.100	4.10 ± 0.100	4.10 ± 0.100	4.10 ± 0.100	4.10 ± 0.100
N_2 flushing (MAP)	P_3	4.00 ± 0.058	4.00 ± 0.115	4.00 ± 0.115	4.00 ± 0.115	4.00 ± 0.208	4.00 ± 0.153	4.00 ± 0.306
	P_4	4.00 ± 0.058	4.00 ± 0.289	4.00 ± 0.289	4.00 ± 0.289	4.00 ± 0.115	4.00 ± 0.115	4.00 ± 0.115
Vacuum packed	P_3	4.00 ± 0.063	4.00 ± 0.212	4.00 ± 0.361	4.00 ± 0.578	4.00 ± 0.532	4.00 ± 0.252	4.00 ± 0.362
	P_4	4.00 ± 0.063	4.00 ± 0.212	4.00 ± 0.578	4.00 ± 0.682	4.00 ± 0.100	4.00 ± 0.122	4.00 ± 0.311

The data represent the mean ± SD of three replicates; ANOVA Table A.16 is given in annexure; Treatment CD at 5% = P_1 (0.08); P_2 (0.08) ; Storage CD at 5% =P_1 (0.08) ; P_2 (0.08) ; P_3 =Combination film, P_4 = autoclavable polythene ; MAP=Modified atmosphere packaging

comparative assessment. However, the only report available suggested a moisture content of 3.4% in a dehydration meat product known as 'Shredded pork' (Ockerman et al.,1999). The low moisture content of meat powder suppresses the growth of microorganisms to a significant extent even upon storage under ambient conditions. It is reported that at the temperature range of 0 to 20°C, the water holding capacity of meat decreases with increasing temperature (Wierbicki and Deatherage, 1958), presumably due to effect on the sarcosplasmic proteins. However, between 20 to 30°C, no change in the degree of hydration occurs, and between 30 to 40°C the polypeptide chains in the muscle proteins unfold and new hydration bonds form, resulting in slight fall in the degree of hydration at the iso-electric point. Between 40 to 50 °C, however, there is a loss of water holding capacity (WHC), which is associated with corresponding diminution in the titrable acidity groups. Between 50 to 80°C, the WHC further decreases though it is less marked and the loss of acidic group continues. Above 80°C, free H_2S begins to form and increases with increasing temperature (Tilgner, 1958). The loss of pre acidic groups explains the considerable rise in the pH of meat. The iso-electric point of the muscle also changes to higher pH values, thus tending to offset the increase in WHC, (Hamm, 1960). The collagen of associated connective tissues also changes as the temperature is raised and increases the WHC of the meat. (Hamm,1960). Moreover, the powdering of meat eases the surface area and thus reduces the moisture content.

4.2.1.3. pH

Table 4.21 presents the data pertaining to pH of meat powder. The data suggest no significant change in the pH of the control verses treated meat powder. However, a remarkable difference in pH value was noticed between raw meat (pH 5.39 ± 0.07) and meat powder (6.03 ± 0.12). Drying and subsequent powdering of meat do not alter the pH of meat. Treatments of meat before drying and powdering

Table 4.21 Effect of treatments on pH of buffalo meat powder during storage of 120 days at ambient temperature.

Samples		pH as a function of storage time (days)						
		0	20	40	60	80	100	120
Control (Untreated)	P_3	6.03 ± 0.12	6.07 ± 0.15	6.03 ± 0.12	6.03 ± 0.12	5.97 ± 0.12	5.97 ± 0.12	5.93 ± 0.06
	P_4	6.03 ± 0.12	6.03 ± 0.12	6.07 ± 0.15	6.07 ± 0.15	5.97 ± 0.12	5.93 ± 0.06	5.93 ± 0.06
Natural products / Preservative								
Clove	P_3	6.00 ± 0.17	6.00 ± 0.00	6.00 ± 0.17	6.00 ± 0.27	5.90 ± 0.17	5.93 ± 0.12	5.93 ± 0.12
	P_4	6.00 ± 0.17	6.00 ± 0.27	6.00 ± 0.20	6.00 ± 0.17	5.93 ± 0.12	5.93 ± 0.12	5.90 ± 0.17
Turmeric	P_3	6.00 ± 0.17	6.00 ± 0.00	6.00 ± 0.17	6.00 ± 0.27	6.00 ± 0.27	6.00 ± 0.27	5.97 ± 0.23
	P_4	6.00 ± 0.17	6.00 ± 0.27	6.00 ± 0.20	6.00 ± 0.17	6.00 ± 0.27	6.00 ± 0.17	5.97 ± 0.15
Potassium.sorbate	P_3	6.03 ± 0.12	6.07 ± 0.15	6.03 ± 0.06	6.03 ± 0.12	6.03 ± 0.06	6.03 ± 0.12	6.03 ± 0.06
	P_4	6.03 ± 0.12	6.03 ± 0.12	6.07 ± 0.06	6.07 ± 0.15	6.03 ± 0.12	6.03 ± 0.06	6.03 ± 0.06
Packaging methods								
CO_2 flushing (MAP)	P_3	6.10 ± 0.10	6.13 ± 0.06	6.10 ± 0.10	6.10 ± 0.10	6.13 ± 0.15	6.10 ± 0.17	6.10 ± 0.17
	P_4	6.10 ± 0.10	6.10 ± 0.10	6.13 ± 0.06	6.13 ± 0.15	6.10 ± 0.10	6.13 ± 0.06	6.13 ± 0.06
N_2 flushing (MAP)	P_3	6.10 ± 0.10	6.13 ± 0.06	6.10 ± 0.10	6.10 ± 0.10	6.13 ± 0.15	6.10 ± 0.17	6.10 ± 0.17
	P_4	6.10 ± 0.10	6.10 ± 0.10	6.13 ± 0.06	6.13 ± 0.15	6.07 ± 0.06	6.03 ± 0.06	5.97 ± 0.06
Vacuum packed	P_3	6.10 ± 0.17	6.13 ± 0.06	6.10 ± 0.17	6.10 ± 0.10	6.10 ± 0.17	6.10 ± 0.17	6.10 ± 0.17
	P_4	6.10 ± 0.10	6.10 ± 0.10	6.13 ± 0.06	6.10 ± 0.17	6.10 ± 0.10	6.13 ± 0.06	6.13 ± 0.06

The data represent the mean ± SD of three replicates ; ANOVA Table A.17 is given in annexure ; Treatment CD at 5%=P_1 (0.072) ; P_2 (0.066) ; Storage CD at 5% =P_1 (0.072) ; P_2 (0.066) ; P_3 =Combination film, P_4 = autoclavable polythene ; MAP=Modified atmosphere packaging

also have an insignificant effect on pH of meat. The increase in pH of meat on drying/dehydration at 40 to 80 °C may be attributed to loss in water holding capacity. The loss of titrable acidic groups cause the rise in pH of meat (Lawrie, 1981, Hamm,1960).

The packaging material also had no significant effect on pH of meat powder. Since, both the packaging materials used in this study provides airtight and moisture proof conditions. The oxidative changes are not expected to occur. However, the non-oxidative change, whether enzymatic or chemical may occur during storage, mainly due to Millard reaction (Henrickson et al., 1955; Sharp, 1957) wherein carbonyl groups of reducing sugars non-enzymatically react with the amino groups of proteins and amino acid. The rate of non-enzymatic browning is known affect the pH.

4.2.1.4 Protein Content

The raw buffalo meat exhibit protein content of 19.32 %. However, transformation of meat to meat powder results in substantial dehydration, which results in concentration of proteinaceous substances in meat. The results in Table 4.22 indicate the protein content of 67.10% in control on day 1. Storage of control upto 120 days exhibited significant decrease in the protein content. The data suggest protein degradation with storage time. Rahman et al. (2005) have also suggested proteolysis as a main factor for reduction in protein content of dehydration meat during storage. Nevertheless, the meat powder treated with the natural and synthetic preservatives showed insignificant effect on protein contents. The two-way ANOVA also revealed the overall effect of treatments on protein contents of meat powder as insignificant, which indicates the protective role of spices on proteins. It is reported that most of the spices also stimulate digestive enzymes such as lipase, amylase and proteases, which play a crucial role in digestion (Platel and Srinivason, 2000). Some of these spices were also found to enhance the activities of terminal digestive

Table 4.22 Effect of treatments on protein content of buffalo meat powder during storage of 120 days at ambient temperature.

Samples		Protein content (%) as a function of storage time (days)						
		0	20	40	60	80	100	120
Control (Untreated)	P_3	67.10 ± 0.45	66.85 ± 0.38	66.86 ± 0.38	66.80 ± 0.32	66.80 ± 0.32	66.78 ± 0.26	66.78 ± 0.32
	P_4	67.10 ± 0.45	66.87 ± 0.38	66.80 ± 0.26	66.82 ± 0.52	66.81 ± 0.49	66.77± 0.32	66.75± 0.32
Natural products / Preservative								
Clove	P_3	67.00 ± 0.26	67.00 ± 0.36	67.00 ± 0.26	66.97 ± 0.40	67.00 ± 0.26	66.97 ± 0.40	67.00 ± 0.46
	P_4	67.00 ± 0.26	66.97 ± 0.40	66.97 ± 0.21	66.97 ± 0.40	66.97 ± 0.40	66.97 ± 0.40	67.00 ± 0.46
Turmeric	P_3	66.93 ± 0.32	66.93 ± 0.40	66.90 ± 0.32	66.90 ± 0.44	66.93 ± 0.32	66.90 ± 0.44	66.87 ± 0.55
	P_4	66.93 ± 0.32	66.90 ± 0.44	66.90 ± 0.26	66.90 ± 0.44	66.90 ± 0.44	66.90 ± 0.44	66.87 ± 0.55
Potassium.sorbate	P_3	67.07 ± 0.50	67.15 ± 0.57	67.10 ± 0.45	67.18 ± 0.57	67.13 ± 0.45	67.13 ± 0.45	67.18 ± 0.57
	P_4	67.07 ± 0.50	67.10 ± 0.45	67.17 ± 0.56	67.18 ± 0.57	67.14 ± 0.45	67.18 ± 0.55	67.10 ± 0.45
Packaging methods								
CO_2 flushing (MAP)	P_3	67.10 ± 0.45	67.13 ± 0.45	67.13 ± 0.45	67.13 ± 0.45	67.13 ± 0.45	67.33 ± 0.55	67.13 ± 0.45
	P_4	67.10 ± 0.45	67.23 ± 0.57	67.13 ± 0.45	67.13 ± 0.45	67.33 ± 0.55	67.23 ± 0.57	67.17 ± 0.55
N_2 flushing (MAP)	P_3	67.10 ± 0.45	67.12 ± 0.45	67.18± 0.55	67.18 ± 0.55	67.16± 0.51	67.16 ± 0.45	67.16 ± 0.55
	P_4	67.10 ± 0.45	67.18± 0.55	67.10 ± 0.41	67.17 ± 0.55	67.17± 0.57	67.17 ± 0.55	67.17 ± 0.55
Vacuum packed	P_3	67.10 ± 0.45	67.30 ± 0.56	67.27 ± 0.45	67.23 ± 0.57	67.33 ± 0.55	67.27 ± 0.57	67.23 ± 0.57
	P_4	67.10 ± 0.45	67.23 ± 0.57	67.30 ± 0.56	67.20 ± 0.56	67.23 ± 0.57	67.17 ± 0.55	67.27 ± 0.59

The data represent the mean ± SD of three replicates; ANOVA Table A.18 is given in annexure; Treatment CD at 5% = P_1 (0.14) ; P_2 (0.14) ; Storage CD at 5% =P_1 (0.14) ; P_2 (0.14) ; P_3 =Combination film, P_4 = autoclavable polythene ; MAP=Modified atmosphere packaging

enzymes of small intestinal mucosa (Platel and Srinivason, 1996, 2001, Sharathchandra et al., 1995). Besides, the spices also stimulate bile acid production by the liver (Bhat et al., 1984). Thus, the supplementation of spices to meat powder not only help protect the protein but also its reconstituted products may accelerate digestion and reduce the food transit time in the gastrointestinal treat, as also suggested by Platel and Srinivason (2001).

Similarly the two packaging materials viz combination film and autoclavable polythene had insignificant ($P>0.05$) effect on powder's protein value, under the atmospheric packaging, modified atmosphere packaging (CO_2 / N_2 flushing) and vacuum packaging condition, as evident from the CD value shown in Table 4.22

Overall, a highly significant increase in protein content of meat powder as compared to protein value of raw meat may be attributed to denaturation of proteins due to heat treatment during drying and subsequent powdering. Drying reduces the water holding capacity of meat, presumably by influencing the sarcoplasmic proteins (Wierbickl and Deatherage,1958). At dehydration temperature of above 40 °C, marked changes in hydrability occurs and drying at temperatures between 40 to 50 °C, the loss in water holding capacity is associated with diminution in the titrable acidic groups.

4.2.1.5. Fat Content

Table 4.23 presents the values of fat content of developed meat powders. In comparison to the fat content value of 10.29 % of raw meat, the fat content of control sample of meat powder, on the day 1 was 11.77 %. The fat content of treated meat powder ranged between 11.80 % to 12.07 %. The data showed that the dehydration and subsequent powdering of meat significantly ($P<0.05$) increased the fat content of meat product. No significant effect of treatment of meat with potassium sorbate

Table 4. 23 Effect of treatments on fat content of buffalo meat powder during storage of 120 days at ambient temperature.

Samples		Fat content (%) as a function of storage time (days)						
		0	20	40	60	80	100	120
Control (Untreated)	P_3	11.77 ± 0.21	11.77 ± 0.21	11.73 ± 0.15	11.73 ± 0.15	11.70 ± 0.10	11.60 ± 0.10	11.53 ± 0.12
	P_4	11.77 ± 0.21	11.77 ± 0.15	11.70 ± 0.10	11.70 ± 0.10	11.63 ± 0.06	11.57 ± 0.06	11.50 ± 0.00
Natural products / Preservative								
Clove	P_3	12.03 ± 0.06	12.07 ± 0.06	12.03 ± 0.06	12.03 ± 0.06	12.03 ± 0.06	12.03 ± 0.06	12.00 ± 0.17
	P_4	12.03 ± 0.06	12.00 ± 0.17	12.07 ± 0.12	12.00 ± 0.17	12.03 ± 0.06	12.07 ± 0.06	12.03 ± 0.06
Turmeric	P_3	12.07 ± 0.13	12.07 ± 0.06	12.03 ± 0.06	12.07 ± 0.12	12.00 ± 0.00	12.07 ± 0.12	12.00 ± 0.00
	P_4	12.07 ± 0.13	12.00 ± 0.00	12.07 ± 0.12	12.07 ± 0.06	12.03 ± 0.06	12.07 ± 0.06	12.03 ± 0.06
Potassium sorbate	P_3	11.80 ± 0.10	11.80 ± 0.30	11.80 ± 0.30	11.80 ± 0.30	11.77 ± 0.12	11.77 ± 0.12	11.70 ± 0.10
	P_4	11.80 ± 0.10	11.80 ± 0.10	11.80 ± 0.10	11.80 ± 0.10	11.73 ± 0.12	11.70 ± 0.10	11.70 ± 0.10
Packaging methods								
CO_2 flushing (MAP)	P_3	11.77 ± 0.21	11.83 ± 0.21	11.73 ± 0.15	11.77 ± 0.10	11.73 ± 0.10	11.73 ± 0.06	11.73 ± 0.12
	P_4	11.77 ± 0.21	11.73 ± 0.15	11.80 ± 0.10	11.73 ± 0.10	11.73 ± 0.15	11.73 ± 0.10	11.67 ± 0.10
N_2 flushing (MAP)	P_3	11.73 ± 0.06	11.77 ± 0.45	11.80 ± 0.20	11.73 ± 0.25	11.73 ± 0.25	11.77 ± 0.25	11.73 ± 0.23
	P_4	11.73 ± 0.06	11.77 ± 0.21	11.77 ± 0.21	11.73 ± 0.23	11.77 ± 0.45	11.73 ± 0.25	11.73 ± 0.25
Vacuum packed	P_3	11.73 ± 0.25	11.77 ± 0.45	11.73 ± 0.25	11.73 ± 0.25	11.77 ± 0.25	11.77 ± 0.25	11.77 ± 0.25
	P_4	11.73 ± 0.25	11.77 ± 0.21	11.77 ± 0.21	11.73 ± 0.23	11.77 ± 0.45	11.73 ± 0.25	11.77 ± 0.25

The data represent the mean ± SD of three replicates; ANOVA Table A.19 is given in annexure; Treatment CD at 5% = P_1 (0.10); P_2 (0.09) ; Storage CD at 5% =P_1 (0.10) ; P_2 (0.09) ; P_3 =Combination film, P_4 = autoclavable polythene ; MAP=Modified atmosphere packaging

was observed as compared to control. However, a marginal increase in fat content was noticed upon treatment with clove or turmeric.

The storage of meat powder in combination film and autoclavable polyethylene for 120 days at ambient conditions by atmospheric packaging did not significantly affect the fat content. Also, with different packaging materials, an insignificant change in fat values was noticed after 120 days storage.

4.2.1.6 TBA

TBA analysis of various treated meat powder was also carried out and the results are presented in Table 4.24. In both the packaging materials, the effect of treatments and storage on TBA number was not significant ($P<0.05$).

In fresh condition, TBA number of controlled meat powder on day 1was 0.29 mg/kg. However, during 120 days storage, the TBA number increased with increasing time and reached to value of 1.29 mg/kg in combination film and upto 1.31 mg/kg in autoclavable film on 120^{th} day of storage. Such a high TBA number indicated that the product crossed the threshold level of rancidity (Ockerman, 1985). In this reference Toldra, (1998) had reported that TBA number increases due to degradation of fatty acid by lipase enzyme and affects the odour score value.

In case of clove and turmeric treated meat powder, the TBA numbers were 0.21 and 0.16, respectively in fresh condition. During storage, the TBA numbers of both clove and turmeric treated powder showed an insignificant increase.

In case of potassium sorbate treated meat powder, the TBA numbers significantly increased with increasing period of storage of 120 days from an initial value of 0.22 to 0.37 mg/kg in combination film and to 0.44 mg/kg in autoclaveable polythene. The samples packed in combination and autoclavable

Table 4.24 Effect of treatments on TBA numbers of buffalo meat powder during storage of 120 days at ambient temperature.

Samples		TBA numbers (mg/kg) as a function of storage time (days)						
		0	20	40	60	80	100	120
Control (Untreated)	P_3	0.29 ± 0.07	0.29 ± 0.07	0.31 ± 0.06	0.47 ± 0.09	0.62 ± 0.09	0.93 ± 0.07	1.29 ± 0.63
	P_4	0.29 ± 0.07	0.33 ± 0.06	0.34 ± 0.03	0.52 ± 0.05	0.67 ± 0.02	1.19 ± 0.63	1.31 ± 0.08
Natural products / Preservative								
Clove	P_3	0.21 ± 0.01	0.22 ± 0.01	0.21 ± 0.01	0.23 ± 0.01	0.26 ± 0.02	0.33 ± 0.02	0.33 ± 0.02
	P_4	0.21 ± 0.01	0.22 ± 0.01	0.22 ± 0.03	0.26 ± 0.03	0.30 ± 0.01	0.35 ± 0.03	0.39 ± 0.06
Turmeric	P_3	0.16 ± 0.01	0.19 ± 0.03	0.21 ± 0.08	0.21 ± 0.08	0.24 ± 0.02	0.32 ± 0.01	0.32 ± 0.01
	P_4	0.16 ± 0.01	0.19 ± 0.03	0.22 ± 0.01	0.23 ± 0.01	0.23 ± 0.03	0.32 ± 0.04	0.33 ± 0.03
Potassium.sorbate	P_3	0.22 ± 0.04	0.29 ± 0.05	0.30 ± 0.09	0.30 ± 0.09	0.31 ± 0.08	0.34 ± 0.04	0.37 ± 0.15
	P_4	0.22 ± 0.04	0.31 ± 0.01	0.34 ± 0.04	0.34 ± 0.04	0.34 ± 0.04	0.34 ± 0.04	0.44 ± 0.07
Packaging methods								
CO_2 flushing (MAP)	P_3	0.21 ± 0.03	0.13 ± 0.09	0.14 ± 0.01	0.19 ± 0.01	0.21 ± 0.02	0.25 ± 0.03	0.37 ± 0.04
	P_4	0.21 ± 0.03	0.16 ± 0.04	0.15 ± 0.05	0.27 ± 0.02	0.34 ± 0.05	0.40 ± 0.03	0.46 ± 0.03
N_2 flushing (MAP)	P_3	0.21 ± 0.03	0.15 ± 0.04	0.13 ± 0.06	0.19 ± 0.02	0.19 ± 0.04	0.22 ± 0.05	0.32 ± 0.05
	P_4	0.21 ± 0.03	0.12 ± 0.02	0.15 ± 0.06	0.26 ± 0.04	0.32 ± 0.04	0.40 ± 0.032	0.44 ± 0.05
Vacuum packed	P_3	0.21 ± 0.03	0.16 ± 0.04	0.14 ± 0.01	0.19 ± 0.01	0.21 ± 0.02	0.29 ± 0.02	0.29 ± 0.02
	P_4	0.21 ± 0.03	0.11 ± 0.01	0.15 ± 0.05	0.27 ± 0.02	0.29 ± 0.07	0.29 ± 0.02	0.32 ± 0.02

The data represent the mean ± SD of three replicates; ANOVA Table A.20 is given in annexure ; Treatment CD at 5% = P_1 (0.05) ; P_2 (0.05) ; Storage CD at 5% =P_1 (0.05) ; P_2 (0.05) ; P_3 =Combination film, P_4 = autoclavable polythene ; MAP=Modified atmosphere packaging

film under different packaging systems namely, CO_2, N_2-flushing and vacuum-packing a showed increase in the TBA numbers. Overall, the vacuum packaging appeared to be the best for long-term storage of meat powder.

4.2.1.7. Ash content

The ash content of raw buffalo meat was determined to be 1.0 %. However, after being processing to meat powder, the ash content on day 1 was assessed as 3.03 % (Table 4.25). Pre-treatment of meat with clove, turmeric or potassium sorbate either showed no change or insignificant increase (by 0.04%) in ash content due to mineral contents of added preservatives (spices or salts). The ash contents of meat powder during storage did not change and also no changed in ash content was observed in meat powder packaged in different packaging materials.

4.2.2. Microbiological characteristics of meat powder

The microbial quality of meat powder was periodically assessed during 120 days of storage at ambient temperature. Certain important indicators of food contamination viz. total plate count (TPC), coliforms, proteolytic, lipolytic, *Staphylococcus*, and yeast and mold (Y&M) counts were recorded. The data are reported in Table 4.26 to 4.30 and discussed as follow:

4.2.2.1. Total plate count (TPC)

Table 4.26 shows the TPC of untreated control and treated meat powders. The results indicate low initial TPC value 1.37 x 10^3 CFU/g in untreated control powder as compared to raw meat. The TPC of buffalo meat obtained by traditional slaughtering and processing in the local slaughter units has been reported to be in the range of 4.82 to 5.49 log CFU cm^2 (Ziauddin et al., 1994). The reduction in TPC counts of meat powder may be to due exposure to high temperature during preparatory process and consequent dehydration of the meat. Even the mild heat treatment of 60°C has been

Table 4.25 Effect treatments on ash content of buffalo meat powder during storage of 120 days at ambient temperature.

Samples		Ash content (%) as a function of storage time (days)						
		0	20	40	60	80	100	120
Control (Untreated)	P_3	3.03 ± 0.10	3.03 ± 0.10	3.03 ± 0.06	3.03 ± 0.10	3.03 ± 0.10	3.03 ± 0.10	3.03 ± 0.10
	P_4	3.03 ± 0.10	3.03 ± 0.10	3.03 ± 0.10	3.03 ± 0.06	3.03 ± 0.10	3.03 ± 0.06	3.03 ± 0.06
Natural products / Preservative								
Clove	P_3	3.07 ± 0.07	3.07 ± 0.06	3.07 ± 0.07	3.07 ± 0.07	3.07 ± 0.07	3.07 ± 0.07	3.07 ± 0.07
	P_4	3.07± 0.07	3.07 ± 0.07	3.07 ± 0.06	3.07 ± 0.07	3.07 ± 0.08	3.07 ± 0.07	3.07 ± 0.07
Turmeric	P_3	3.07 ± 0.06	3.07 ± 0.06	3.07 ± 0.06	3.07 ± 0.07	3.07 ± 0.06	3.07 ± 0.06	3.07 ± 0.07
	P_4	3.07 ± 0.06	3.07 ± 0.07	3.07 ± 0.06	3.07 ± 0.06	3.07 ± 0.07	3.07 ± 0.06	3.07 ± 0.07
Potassium Sorbate	P_3	3.03 ± 0.06	3.03 ± 0.05	3.02 ± 0.04	3.00 ± 0.10	3.00 ± 0.10	3.03 ± 0.05	3.03 ± 0.05
	P_4	3.03 ± 0.06	3.03 ± 0.06	3.03 ± 0.06	3.03 ± 0.10	3.03 ± 0.04	3.03 ± 0.06	3.00 ± 0.05
Packaging methods								
CO_2 flushing (MAP)	P_3	3.03 ± 0.15	3.03 ± 0.06	3.00 ± 0.06	3.03 ± 0.15	3.03 ± 0.00	3.03 ± 0.00	3.03 ± 0.15
	P_4	3.03 ± 0.15	3.03 ± 0.00	3.00± 0.06	3.03 ± 0.00	3.01 ± 0.06	3.00 ± 0.06	3.00 ± 0.06
N_2 flushing (MAP)	P_3	3.03 ± 0.15	3.03 ± 0.06	3.02 ± 0.06	3.03 ± 0.15	3.03 ± 0.15	3.03 ± 0.15	3.03 ± 0.15
	P_4	3.03 ± 0.15	3.03± 0.15	3.02 ± 0.15	3.03 ± 0.00	3.03 ± 0.06	3.03 ± 0.06	3.03 ± 0.06
Vacuum packed	P_3	3.03 ± 0.06	3.03 ± 0.15	3.03 ± 0.06	3.02 ± 0.06	3.01 ± 0.06	3.01 ± 0.06	3.03 ± 0.00
	P_4	3.03 ± 0.06	3.02 ± 0.15	3.03 ± 0.15	3.03 ± 0.15	3.03 ± 0.00	3.03 ± 0.06	3.03 ± 0.06

The data represent the mean ± SD of three replicates; ANOVA Table A.21 is given in annexure ; Treatment CD at 5% = P_1 (0.04) ; P_2 (0.04) ; Storage CD at 5% =P_1 (0.04) ; P_2 (0.04) ; P_3 =Combination film, P_4 = autoclavable polythene ; MAP=Modified atmosphere packaging

Table 4.26 Effect of treatments on TPC counts of buffalo meat powder during storage of 120 days at ambient temperature.

Samples		TPC ($x10^3$CFU/g) as a function of storage time (days)						
		0	20	40	60	80	100	120
Control (untreated)	P_3	1.37 ± 0.205	1.58 ± 0.228	1.74 ± 0.023	2.24 ± 0.031	2.48 ± 0.010	3.44 ± 0.324	4.48 ± 0.219
	P_4	1.37 ± 0.205	1.64 ± 0.056	1.84 ± 0.052	2.33 ± 0.078	2.67 ± 0.766	3.70 ± 0.095	5.44 ± 0.324
Natural products / Preservative								
Clove	P_3	ND	ND	ND	ND	ND	ND	ND
	P_4	ND	ND	ND	ND	ND	ND	ND
Turmeric	P_3	ND	ND	ND	ND	ND	ND	ND
	P_4	ND	ND	ND	ND	ND	ND	ND
Potassium Sorbate	P_3	0.19 ± 0.035	0.27 ± 0.021	0.37 ± 0.021	0.54 ± 0.056	0.67 ± 0.120	0.91 ± 0.102	1.01 ± 0.231
	P_4	0.19 ± 0.035	0.27 ± 0.021	0.47 ± 0.107	0.74 ± 0.056	0.84 ± 0.046	1.11 ± 0.238	0.87 ± 0.669
Packaging methods								
CO_2 flushing (MAP)	P_3	ND	ND	ND	ND	ND	ND	ND
	P_4	ND	0.54 ± 0.144	0.71± 0.064	0.94 ± 0.053	1.61±0.100	2.85±0.020	4.00 ± 0.538
N_2 flushing (MAP)	P_3	ND	ND	ND	ND	ND	ND	ND
	P_4	ND	ND	0.18 ± 0.061	0.48 ± 0.045	1.54 ± 0.303	2.34 ± 0.055	7.08 ± 0.361
Vacuum packed	P_3	ND	ND	ND	ND	ND	ND	ND
	P_4	ND	ND	ND	ND	ND	ND	ND

The data represent the mean ± SD of three replicates; ANOVA Table A.22 is given in annexure ; Treatment CD at 5% = P_1 (0.03); P_2 (0.11) ; Storage CD at 5% =P_1 (0.03) ; P_2 (0.11) ; P_3 =Combination film, P_4 = autoclavable polythene ; ND= not detected
MAP=Modified atmosphere packaging

reported to lower the microbial load (Sachindra et al.,1998). However, the data show that the TPC values of control meat powder increased with time during storage at ambient condition. As compared to the initial count of 1.37 x 10^3 CFU/g, the bacterial population after 120 days of storage increased to 4.48 x 10^3 CFU/g and 5.44 x 10^3 CFU/g in combination film and autoclaveable polythene, respectively. These results suggest that the autoclavable polythene does not serve as a suitable packaging material as the bacterial growth have occurred in this packaging material. The growth increases substantially upto 120 days even when packaged under CO_2 and N_2 flushing. However, the vacuum packed meat powder remained microbiologically stable with no detectable count even after 120 days of storage. The results explicitly demonstrated that vacuum packaging of meat powder is most suitable amongst all treatments for increasing the shelf-life of the product. Furthermore, the treatment of meat powder with both the natural and synthetic preservatives yielded very stable products. The meat powder packed in different packaging materials after treatment with 2% clove, turmeric and potassium sorbate remained contamination free upto 120 days storage. The treatment with test preservatives substantially reduced the microbial load, which is attributed to the antimicrobial activity of the natural spices.

The data clearly demonstrated that the meat stored in powdered form could be maintained at ambient temperature upto 120 days without any spoilage and / or change in organoleptic characteristics of the products prepared with the stored meat powder. The powder was used for Kabab preparation after reconstitution. The organoleptic data of the Kababs are shown in Table 4.27 The data revealed that the kababs prepared from meat powder were very well accepted and placed in "most likely" category based on the hedonic parameters of assessment. This is presumably due to low initial count of bacteria in dehydrated meat powder. Microbial

Table 4. 27 Organoleptic properties of kabab developed from meat powder given different treated pre treatments.

Samples		Colour	Odour	Texture	Taste	Palatability
Control (untreated)	P_3	5.33 ± 0.58	4.70 ± 0.58	6.00 ± 1.00	5.70 ± 0.58	5.32 ± 0.58
	P_4	5.33 ± 0.58	4.70 ± 1.00	6.00 ± 1.53	5.71 ± 1.00	5.33 ± 0.58
Natural products / Preservative						
Clove	P_3	7.33 ± 1.15	7.70 ± 0.58	6.71 ± 1.15	6.32 ± 0.58	6.32 ± 0.58
	P_4	7.33 ± 1.53	7.70 ± 1.16	6.71 ± 1.53	6.33 ± 1.00	6.33 ± 1.16
Turmeric	P_3	7.33 ± 1.53	7.33 ± 1.15	6.72 ± 1.53	6.00 ± 1.00	6.32 ± 1.15
	P_4	7.33 ± 0.53	7.33 ± 0.58	6.72 ± 0.58	6.00 ± 1.16	6.33 ± 0.58
Potassium sorbate	P_3	6.33 ± 0.58	7.32 ± 1.15	6.72 ± 0.58	7.33 ± 0.58	6.71 ± 0.58
	P_4	6.33 ± 0.58	7.32 ± 0.58	6.72 ± 0.58	6.00 ± 1.16	6.33 ± 0.58
Packaging methods						
CO_2 flushed (MAP)	P_3	7.70 ± 0.58	7.00 ± 1.00	6.71 ± 1.53	8.00 ± 1.00	7.71 ± 0.58
	P_4	7.70 ± 0.58	7.00 ± 0.58	6.67 ± 1.53	8.00 ± 0.56	7.67 ± 0.58
N_2 flushed (MAP)	P_3	7.70 ± 0.58	8.72 ± 0.58	6.71 ± 1.53	7.72 ± 0.58	7.71 ± 0.58
	P_4	7.70 ± 0.58	8.72 ± 0.58	6.72 ± 0.53	7.67 ± 0.58	7.67 ± 0.58
Vacuum packaging	P_3	7.70 ± 0.58	8.71 ± 0.58	6.72 ± 0.53	8.33 ± 0.58	7.70 ± 0.58
	P_4	7.70 ± 0.58	8.71 ± 1.16	6.67 ± 0.53	8.33 ± 0.58	7.67 ± 0.58

P_3 =Combination film, P_4 = Autoclavable polythene, MAP=Modified atmosphere packaging, The data presents each mean score value ± SD of three replicate

contamination is largely responsible for meat spoilage (Ingram and Dainty, 1971; Gill and Newton, 1980; Dainty, et al., 1975). Slaughter practices and environmental conditions in different geographical regions have an impact on the microbial ecology of meat (Nottingham, 1982; Rao et al., 1998). This study has demonstrated the microbes free preparation and storage of raw meat in powdered form at room temperature, which otherwise is difficult to preserve even at sub -zero temperatures.

4.2.2.2. The Coliform and *Staphylococcal* counts

Assessment of common bacterial contaminants in powdered meat shows undetectable level of coliforms (data not shown). The absence of *Escherichia* group indicates the hygienic processing of meat. Rao and Ramesh (1992) have also reported low incidence of *E.coli* on sheep carcasses. However, Ziauddin (1988) has observed the higher incidence of *E.coli* (15%), *Enterobacter* (12%) and *Staphylococcus* (37%) in buffalo meat. However, the persistence of *Staphylococcus* group of bacteria has been noticed in meat powder. The data shown in Table 4.28 indicate the presence of *Staphylococcus* on day 1 of the meat powder preparation. Although, the population of *Staphylococcus* determined to be low (0.39×10^3 CFU/g), the resistance of this common inhabitant of meat towards heat and dehydration has been noticed. Indeed, a significant proportion of bacterial population have been killed during meat powder processing. Nevertheless, a fraction of population exhibited the capacity to thrive at extremely low moisture and survived under the harsh treatment conditions. Bacus et al., (1986) have also reported that *Staphylococcus aureus* survives at low a_w levels in dried meat.

Unlike untreated control, the meat powder supplemented with natural preservatives viz. clove (2%) and turmeric (2%) strictly checked the microbial growth. The data in Table 4.28 suggest 'no growth' of *Staphylococcus* in meat powder from day 1 to the end of storage at ambient temperature. The meat powder

Table 4.28 Effect of treatments on *Staphylococcus* count of buffalo meat powder during storage of 120 days at ambient temperature.

Samples		*Staphylococcus* count (x10^3CFU/g) as a function of storage time (days)						
		0	20	40	60	80	100	120
Control (untreated)	P_3	0.39 ± 0.04	0.53 ± 0.08	1.29 ± 0.08	1.48 ± 0.09	3.31 ± 0.35	4.84 ± 0.30	5.08 ± 0.65
	P_4	0.39 ± 0.04	0.69 ± 0.05	1.68 ± 0.12	4.36 ± 0.12	4.92 ± 0.54	6.18 ± 0.04	6.75 ± 0.08
Natural products / Preservative								
Clove	P_3	ND	ND	ND	ND	ND	ND	ND
	P_4	ND	ND	ND	ND	ND	ND	ND
Turmeric	P_3	ND	ND	ND	ND	ND	ND	ND
	P_4	ND	ND	ND	ND	ND	ND	ND
Potassium Sorbate	P_3	ND	ND	ND	ND	ND	ND	0.35 ± 0.10
	P_4	ND	ND	ND	ND	ND	0.42 ± 0.09	0.58 ± 0.08
Packaging methods								
CO_2 flushing (MAP)	P_3	ND	ND	ND	ND	ND	ND	ND
	P_4	ND	ND	ND	ND	ND	ND	ND
N_2 flushing (MAP)	P_3	ND	ND	ND	ND	ND	ND	ND
	P_4	ND	ND	ND	ND	ND	ND	ND
Vacuum packed	P_3	ND	ND	ND	ND	ND	ND	ND
	P_4	ND	ND	ND	ND	ND	ND	ND

The data represent the mean ± SD of three replicates ; ANOVA Table A.23 is given in annexure ; Treatment CD at 5% = P_1 (0.06) ; P_2 (0.04) ; Storage CD at 5% =P_1 (0.06) ; P_2 (0.04) ; P_3 =Combination film, P_4 = autoclavable polythene ; ND= not detected MAP=Modified atmosphere packaging

with antifungal agent potassium sorbate, however showed some growth at 100 and 120 days of storage. The results clearly suggested the antimicrobial effect of spices and demonstrated that a mixture of 2% spice could be sufficient to increase the keepability of meat powder without any refrigeration.

To the best of our knowledge, this is the first report on the preparation and storage of meat in powdered form, which can be stored at ambient temperature upto 120 days using natural spices as preservatives, maintaining a low TBA value and zero *Staphylococcus* count. Such a method of meat preservation exhibits enormous potential of utilizing this perishable food for longer duration besides providing the opportunity of faster reconstitution into customized derivatives. Meat balls and Kababs of varying shapes could be developed from meat powder with minimal microbial contaminants, and without compromising the food quality and taste. Interestingly, the three packaging conditions viz. CO_2 flushing, N_2 flushing and vacuum package in two different packaging materials as combination film and autoclavable polythene have also not supported any microbial growth. The data in Table 4.28 exhibited no *Staphylococcal* growth, which clearly suggest the suitably of both materials for storage of meat powder under ambient conditions.

4.2.2.3 Proteolytic and lipolytic counts

Tables 4.29 and 4.30 show the proteolytic and lipolytic counts in meat powder during storage upto 120 days. The control untreated meat powder shows the presence of proteolytic bacteria. The data in Table 4.29 showed that the initial counts of proteolytic bacteria is relatively low (1.07×10^3 CFU/g). However, the count increases with the time of storage to a maximum extent of 4.74×10^3 and 6.75×10^3 CFU/g in meat powder stored in combination film and autoclavable polythene, respectively.

Table 4.29 Effect of treatments on proteolytic count of buffalo meat powder during storage of 120 days at ambient temperature.

Samples		Proteolytic count (x10^3CFU/g) as a function of storage time (days)						
		0	20	40	60	80	100	120
Control (untreated)	P_3	1.07 ± 0.06	1.43 ± 0.58	1.93 ± 0.76	2.03 ± 0.50	3.28 ± 0.04	3.54 ± 0.07	4.74 ± 0.14
	P_4	1.07 ± 0.06	1.47 ± 0.55	1.97 ± 0.42	3.68 ± 0.12	4.32 ± 0.01	5.25 ± 0.61	6.75 ± 0.08
Natural products / Preservative								
Clove	P_3	ND	ND	ND	ND	ND	ND	ND
	P_4	ND	ND	ND	ND	ND	ND	ND
Turmeric	P_3	ND	ND	ND	ND	ND	ND	ND
	P_4	ND	ND	ND	ND	ND	ND	ND
Potassium Sorbate	P_3	ND	ND	ND	ND	ND	ND	ND
	P_4	ND	ND	ND	ND	ND	ND	ND
Packaging methods								
CO_2 flushing (MAP)	P_3	ND	ND	ND	ND	ND	ND	ND
	P_4	ND	ND	ND	ND	0.46 ± 0.09	0.51 ± 0.13	1.65 ± 0.15
N_2 flushing (MAP)	P_3	ND	ND	ND	ND	ND	ND	ND
	P_4	ND	ND	ND	ND	ND	0.36 ± 0.02	0.81 ± 0.38
Vacuum packed	P_3	ND	ND	ND	ND	ND	ND	ND
	P_4	ND	ND	ND	ND	ND	ND	ND

The data represent the mean ± SD of three replicates ; ANOVA Table A.24 is given in annexure ; Treatment CD at 5% = P_1 (0.04) ; P_2 (0.02) ; Storage CD at 5% =P_1 (0.04) ; P_2 (0.02) ; P_3 =Combination film, P_4 = autoclavable polythene ; ND= not detected
MAP=Modified atmosphere packaging

Table 4 .30 Effect of treatments on lipolytic count of buffalo meat powder during storage of 120 days at ambient temperature.

Samples		lipolytic count (x10^3CFU/g) count (x10^3CFU/g) as a function of storage time (days)						
		0	20	40	60	80	100	120
Control (untreated)	P_3	ND	ND	0.22 ± 0.06	0.46 ± 0.03	1.15 ± 0.03	1.40 ± 0.03	1.63 ± 0.10
	P_4	ND	ND	0.28 ± 0.02	0.64 ± 0.07	1.28 ± 0.02	1.34 ± 0.08	1.90 ± 0.37
Natural products / Preservative								
Clove	P_3	ND	ND	ND	ND	ND	ND	ND
	P_4	ND	ND	ND	ND	ND	ND	ND
Turmeric	P_3	ND	ND	ND	ND	ND	ND	ND
	P_4	ND	ND	ND	ND	ND	ND	ND
Potassium Sorbate	P_3	ND	ND	ND	ND	ND	ND	ND
	P_4	ND	ND	ND	ND	ND	ND	ND
Packaging methods								
CO_2 flushing (MAP)	P_3	ND	ND	ND	ND	ND	ND	ND
	P_4	ND	ND	ND	ND	ND	ND	ND
N_2 flushing (MAP)	P_3	ND	ND	ND	ND	ND	ND	ND
	P_4	ND	ND	ND	ND	ND	ND	ND
Vacuum packed	P_3	ND	ND	ND	ND	ND	ND	ND
	P_4	ND	ND	ND	ND	ND	ND	ND

The data represent the mean ± SD of three replicates ; Treatment CD at 5% = P_1 (0.04) ; P_2 (0.02) ; Storage CD at 5% =P_1 (0.04) ; P_2 (0.02) ; P_3 =Combination film, P_4 = autoclavable polythene; ND= not detected ; MAP=Modified atmosphere packaging

The presence of lipolytic bacteria has been noticed in untreated control sample. The results indicate that these bacteria appeared after 40 days of storage under ambient conditions. The population of lipolytic bacteria increase from the initial count of 0.22 x 10^3 CFU/g to 1.63 and 1.9 x 10^3 CU/g in combination film and autoclavable polythene, respectively after 120 days of storage (Table 4.30). Although, the proteolytic and lipolytic activities have been reported to reduce with increasing temperature, the storage of meat powder at temperature between 20 ° to 30° C, does promote some proteolysis and lypolysis. However, at temperature above 30 °C, the activity of proteases and lipases are inhibited. The addition of natural preservatives to meat powder viz. clove and turmeric also resulted in growth inhibition of both the proteolytic or lipolytic bacteria upto 120 days of storage, at ambient temperature. Also, the treatment of meat powder with the natural and synthetic preservatives effectively checked the growth of lipolytic bacteria. Nevertheless, the proteolytic bacteria appeared on 80th day of storage in meat powder stored under CO_2-flushing condition and on 100th day under N_2-flushing conditions. The vacuum treatment, with no bacterial growth is therefore, considered to be the most effective method for controlling the over all microbial population, including the proteolytic and lipolytic bacteria. This suggest that the meat powder preserved with spice under vacuum have a longer shelf-life besides maintaining the nutrient and organoleptic quality of meat.

4.2.2.4. Yeast & mold counts

The freshly prepared control untreated meat powder exhibits the yeast and mold population on day 1 at ambient temperature. The initial count of yeast and mold, as determined to be 1.41 x 10^3 CFU/g increased significantly (P>0.05) to the levels of 11.6 and 12.2 x 10^3 CFU/g in combination film and autoclavable polythene, respectively (Table 4.31). Clove (2%) used as natural preservatives has significantly controlled the growth of the yeast and mold during 120 days storage. Another natural

Table 4.31 Effect of treatments on Y&M count of buffalo meat powder during storage of 120 days at ambient temperature.

Samples		Y&M count ($x10^3$CFU/g) as a function of storage time (days)						
		0	20	40	60	80	100	120
Control (untreated)	P_3	1.41 ± 0.17	2.25 ± 0.47	2.76 ± 0.05	3.67 ± 0.49	6.51 ± 0.54	9.11 ± 0.65	11.6 ±0.11
	P_4	1.41 ± 0.17	2.41 ± 0.45	2.83 ± 0.05	3.76 ± 0.11	7.61 ± 0.56	9.94 ± 0.04	12.2 ± 0.85
Natural products / Preservative								
Clove	P_3	ND	ND	ND	ND	ND	ND	ND
	P_4	ND	ND	ND	ND	ND	ND	ND
Turmeric	P_3	ND	ND	ND	ND	ND	ND	0.64 ± 0.13
	P_4	ND	ND	ND	ND	0.44 ± 0.06	0.71 ± 0.11	1.44 ± 0.11
Potassium Sorbate	P_3	ND	ND	ND	ND	ND	ND	ND
	P_4	ND	ND	ND	ND	ND	ND	ND
Packaging methods								
CO_2 flushing (MAP)	P_3	ND	ND	ND	ND	ND	ND	ND
	P_4	ND	ND	ND	0.72 ± 0.08	1.45 ± 0.06	1.59 ± 0.11	2.76 ± 0.05
N_2flushing (MAP)	P_3	ND	ND	ND	ND	ND	ND	ND
	P_4	ND	ND	ND	0.75 ± 0.12	1.58 ± 0.17	3.35 ± 0.02	5.58 ± 0.17
Vacuum packed								
	P_3	ND	ND	ND	ND	ND	ND	ND
	P_4	ND	ND	ND	ND	ND	ND	ND

The data represent the mean ± SD of three replicates ; ANOVA Table A.25 is given in annexure ; Treatment CD at 5% = P_1 (0.11) ; P_2 (0.10) ; Storage CD at 5% =P_1 (0.11) ; P_2 (0.10) ; P_3 =Combination film, P_4 = autoclavable polythene ; ND= not detected ; MAP=Modified atmosphere packaging

preservative (turmeric) used at the same level also prevented the growth of these microorganisms during storage in combination film. However, the growth of yeast and molds appeared in meat powder upon 80 days of storage in autoclavable polythene.

Moreover, the Kababs prepared from the reconstituted powder have also exhibited all desired characteristics acceptable to the panel of experts during organoleptic evaluation. Comparative assessment of the treatment methods of storage revealed that CO_2 flushing and N_2 flushing in autoclavable polythene are not appropriate for storage of meat powder. The fungal population under these two conditions enhanced significantly attaining the levels of 2.76×10^3 and 5.58×10^3 CFU/g on 120^{th} day of storage. On the contrary, the vacuum packed meat powder either in combination film or in autoclavable polythene has been observed to be the most appropriate storage condition for protecting meat powder from fungal contamination.

The systematic study on the bacteriological and fungal assessments of meat powder under different packaging conditions and materials with both the natural and synthetic preservatives provided, the conclusive information related to the (i) profile of microbial population in meat powder (ii) comparable assessment of the natural and synthetic preservatives in terms microbial control (iii) suitabling of packaging conditions for storage of meat powder and (iv) selection of appropriate material for storage at ambient temperature with minimal microbial contamination, without compromising the quality of the meat food. The minimization of microbial contamination is essential in meat handling system in order to delay the spoilage as well as to prevent the associated health hazards.

4.2.3. Organoleptic characteristics of meat powders

4.2.3.1. Colour

Table 4.32 presents the colour scores of meat powders. On the day 1, the colour score of control samples of meat powder was 8.3 rated as liked very much. Treatment of meat after dehydration and powdering with turmeric and potassium sorbate, in comparison to control, did not changed the colour score. On the contrary, in case of treatment with clove, a significant ($P<0.05$) decrease in colour score was noticed. Though the colour score decreased by only 0.6 units but this has affected the quality of powder and the product was rated as 'liked moderately'. Treatments with turmeric and potassium sorbate resulted in pale colour where as a darker colour developed with the clove treatment as compared to control.

Storage of meat powder in combination film and autoclavable polyethylene for 120 days at ambient temperature decreased the colour score significantly ($P<0.05$) in control but no change in colour scores was observed in case of other treatments. This indicated that treatments of meat preservatives like clove and potassium sorbate provided stability to the colour of the powder during storage. The packaging material also did not showed any significant effect on colour scores of treated meat powder during storage, though significantly ($P<0.05$) affected the score in case of storage of control samples.

Comparing the various methods of packaging used for meat powders, the vacuum packaging and CO_2-flushing of powder in combination film were assessed to be the better packaging systems. Out of the two packaging materials, the autoclavable polyethylene film was better as compared to N_2 and CO_2-flushing. This phenomenon may be attributed to presence of oxygen in packaging material in case of atmospheric packaging.

Table 4 .32 Effect of treatments on colour score of buffalo meat powder during storage of 120 days at ambient temperature.

Samples		Colour values as a function of storage time (days)						
		0	20	40	60	80	100	120
Control (untreated)	P_3	8.3 ± 0.58	8.0 ± 0.00	8.0 ± 0.00	8.0 ± 0.00	7.7 ± 0.58	7.3 ± 0.58	7.3 ± 0.58
	P_4	8.3 ± 0.58	7.7 ± 0.58	7.3 ± 0.58	7.0 ± 000	6.7 ± 0.58	6.3 ± 0.58	6.3 ± 0.58
Natural products / Preservative								
Clove	P_3	7.7 ± 0.58	7.7 ± 1.16	7.7 ± 1.16	7.7 ± 0.58	7.7 ± 0.58	7.7 ± 1.16	7.7 ± 1.16
	P_4	7.7 ± 0.58	7.7 ± 1.53	7.7 ± 1.16	7.7 ± 1.16	7.7 ±0.58	7.7 ± 0.58	7.7 ± 0.58
Turmeric	P_3	8.3 ± 0.58	8.3 ± 1.16	8.3 ± 1.16	8.3 ± 0.58	8.3 ± 0.58	8.3 ± 1.16	8.3 ± 1.16
	P_4	8.3 ± 0.58	8.3 ± 0.58	8.3 ± 1.16	8.3 ± 1.16	8.3 ± 0.58	8.3 ± 0.58	8.3 ± 0.58
Potassium sorbate	P_3	8.3 ± 0.58	8.3 ± 0.58	8.3 ± 0.58	8.3 ± 0.58	8.3 ± 0.58	8.3 ± 0.58	8.3 ±0.58
	P_4	8.3 ± 0.58	8.3 ± 0.58	8.3 ± 0.56	8.3 ± 0.58	8.3 ± 0.58	8.3 ± 0.58	8.3 ± 0.58
Packaging methods								
CO_2 flushing (MAP)	P_3	8.7 ± 0.58	8.7 ± 0.58	8.3 ± 0.58	8.3 ± 0.58	8.3 ± 0.58	7.7 ± 1.16	8.3 ± 0.58
	P_4	8.7 ± 0.58	7.7 ± 0.58	7.7 ± 0.58	7.3 ± 0.58	6.7 ± 0.58	6.3 ± 0.58	6.3 ± 0.58
N_2 flushing (MAP)	P_3	8.7 ± 0.58	8.7 ± 0.58	8.7 ± 0.58	8.7 ± 0.58	7.7 ± 0.58	7.7 ± 1.16	7.7 ± 0.58
	P_4	8.7 ± 0.58	8.0 ± 0.00	7.7 ± 0.58	7.7 ± 0.58	6.7 ± 0.58	6.7 ± 0.58	6.7 ± 0.58
Vacuum Packaging	P_3	8.3 ± 0.58	8.3 ± 0.58	8.3 ± 0.58	8.3 ± 0.58	8.0 ± 0.00	8.0 ± 1.00	8.3 ± 0.58
	P_4	8.3 ± 0.58	8.0 ± 0.00	7.7 ± 0.58	7.3 ± 0.58	7.3 ± 0.58	7.0 ± 1.00	7.0 ± 1.00
Treatment CD at 5%	P_3	0.33				**Storage CD at 5%**	P_3	0.33
	P_4	0.35					P_4	0.35

The data represent the mean ± SD of three replicates ; ANOVA Table A.26 is given in annexure ; Treatment CD at 5%= (0.11) ; P_2 (0.10) ; Storage CD at 5% =P_1 (0.11) ; P_2 (0.10) ; P_3 =Combination film, P_4 = autoclavable polythene ; ND= not detected ; MAP=Modified atmosphere packaging

It may be noted that the dark brown colour developed in case of storage of dehydrated meat in the absence of oxygen (i.e. in vacuum and modified atmosphere packaging) could be due to the Millard reaction (Henrickson et al., 1955; Sharp, 1957) where in carbonyl groups of reducing sugar and amino groups of proteins reacts non-enzymatically. However in the presence of oxygen (as in atmosphere packaging) the storage of dehydrated meat causes it to become pale and yellow due to conversion of myoglobin to bile pigments (Lawrie, 1991).

4.2.3.2. Odour

Table 4.33 presents the odour scores of meat powders. The control sample on day 1 obtained the score of 8.33, and was rated as 'liked very much'. The clove treatment of meat after drying and powdering insignificantly affected the odour score due to sweet smell of clove as compared to control treatment and was generally appreciated. However, the treatments with turmeric and potassium sorbate significantly ($P<0.05$) decreased the odour scores as a result of which these two products (in comparison to control samples) were rated as 'liked moderately'. Showing marginal decrease in odour value, potassium sorbate treatment gives a fruity, grassy and chemical smell. Treatment with turmeric also resulted in typical smell due to presence of curcumin, curcuminoids and curcumin oil, which are slightly disliked by many consumers.

The odour score of control powders, stored in two different packaging materials at ambient temperatures for 120 days showed a significant ($P<0.05$) decrease. However, the effect of storage period on odour scores of meat powder, treated with spices or salts was insignificant, suggesting that the treatments helped in preserving the odour of meat powders. Comparing the various methods of packaging used for meat powder it was observed that N_2-flushing and combination film was the best method for storage of meat powder as there was no change in odour score

Table 4.33 Effect of treatments on odour of buffalo meat powder during storage of 120 days at ambient temperature.

Samples		Odour score as a function of storage time (days)						
		0	20	40	60	80	100	120
Control (untreated)	P_3	8.33 ± 0.58	8.00 ± 0.00	8.33 ± 0.58	8.00 ± 1.00	8.00 ± 1.00	7.67 ± 0.58	7.70 ± 0.58
	P_4	8.33 ± 0.58	7.65 ± 0.58	7.67 ± 0.58	7.00 ± 0.00	7.00 ± 0.00	6.67 ± 0.58	6.70 ± 0.58
Natural products / Preservative								
Clove	P_3	8.67 ± 0.58	8.67 ± 0.58	8.67 ± 0.58	8.67 ± 0.58	8.73 ± 0.58	8.73 ± 0.58	8.67 ± 0.58
	P_4	8.67 ± 0.58	8.67 ± 0.58	8.67 ± 0.58	8.67 ± 0.58	8.33 ± 0.58	8.33 ± 0.58	8.33 ± 0.58
Turmeric	P_3	7.33 ± 0.58	7.33 ± 1.53	7.33 ± 0.58	7.33 ± 0.58	7.33 ± 0.58	7.33 ± 0.58	7.33 ± 0.58
	P_4	7.33 ± 0.58	7.33 ± 0.58	7.33 ± 0.58	7.33 ± 0.58	7.33 ± 0.58	7.33 ± 0.58	7.33 ± 0.58
Potassium sorbate	P_3	7.65 ± 0.58	7.70 ± 0.58	7.67 ± 0.58	7.67 ± 0.58	7.33 ± 0.58	7.73 ± 0.56	7.67 ± 0.58
	P_4	7.65 ± 0.58	7.70 ± 0.58	7.33 ± 0.58	7.33 ± 0.58	7.33 ± 0.58	7.33 ± 0.58	7.33 ± 0.58
Packaging methods								
CO_2 flushing (MAP)	P_3	8.33± 0.58	8.33 ± 0.58	8.33 ± 0.58	8.33 ± 0.58	8.33 ± 0.58	7.33 ± 0.58	7.33 ± 0.58
	P_4	8.33 ± 0.58	7.70 ± 0.58	7.33 ± 0.58	7.33 ± 0.58	7.00 ± 0.00	6.73 ± 0.58	6.67 ± 0.58
N_2 flushing (MAP)	P_3	8.33 ± 0.58	8.33 ± 0.58	8.33 ± 0.58	8.33 ± 0.58	8.33 ± 0.58	8.33 ± 0.58	8.33 ± 0.58
	P_4	8.33 ± 0.58	8.00 ± 0.00	7.67 ± 0.58	7.67 ± 0.58	7.70 ± 0.58	7.73 ± 0.58	7.67 ± 0.58
Vacuum Packaging	P_3	8.67 ± 0.58	8.67 ± 0.58	8.67 ± 0.58	8.33 ± 0.58	8.33 ± 0.58	7.00 ± 0.58	7.67 ± 0.58
	P_4	8.67 ± 0.58	8.33 ± 0.58	8.33 ± 0.58	7.33 ± 0.58	7.33 ± 0.58	7.00 ± 1.00	7.00 ± 1.00

The data represent the mean ± SD of three replicates ; ANOVA Table A.27 is given in annexure ; Treatment CD at 5%= (0.35) ; P_2 (0.31) ; Storage CD at 5% =P_1 (0.35) ; P_2 (0.31) ; P_3 =Combination film, P_4 = autoclavable polythene ; ND= not detected ; MAP=Modified atmosphere packaging

during 120 days storage. All other methods of packaging and packaging materials (combination of two) significantly reduced ($P<0.05$) the odour score in following decreasing order: vacuum packaging in combination film > CO_2-flushing in autoclavable film > atmospheric packaging in autoclavable film > CO_2-flushing in combination film or vacuum packaging in combination film > N_2 flushing in autoclavable polyethylene > atmospheric packaging in combination film.

Decrease in odour scores of powder during storage may be attributed to increase in rancidity (TBA number) due to degradation of fatty acids as suggested by Tarladgis et al. (1960). Flushing in N_2 results in restriction of oxygen during storage and helps in maintaining the flavour of dehydrated meat. In case of storage by atmosphere packaging and CO_2 packaging, the presence of oxygen causes a meaty odour development while fat oxidation results in paint like odours as also reported by Lawrie (1991).

4.2.4 Shelf life of hurdle processed meat powder

The meat powder samples packaged in combination film and autoclavable polyethylene showed much lower microbial count during storage under ambient conditions. The treated meat powder samples, irrespective of treatments and packaging materials / methods of packaging exhibited the shelf life of 120 days at ambient conditions. Interestingly, the kababs prepared from 120 days preserved powders from both packaging materials exhibited good organoleptic characteristics with the palatability score of 6.32 to 7.67 on hedonic rating scale. These results clearly suggested the prolong shelf life of the meat powder and its products virtually no sign of spoilage upon storage without refrigeration.

Summary & Conclusions

India is a major producer of buffalo meat with a total out put of about 1.4 million tones, owing to large population (~ 494.4 millions) of this animal. The production of buffalo meat in India contributes to about 50 % of world production, and roughly 85 % of it is being exported from India. However, the meat in this country is mostly produced from aged and unproductive animals and, therefore, it is usually fibrous and tough in texture. Grinding of this kind of meat leads to rapid formation of metmyoglobin with undesirable brown colour and oxidative rancidity, which severely affects the consumer acceptance.

Since, raw meat is highly perishable in nature, it demands immediate processing and preservation. Meat processed through common techniques as freeze dehydration or thermal processing is not only expensive but also adversely affects the quality of meat. These major limitations could be easily surmounted with the simple and inexpensive approach of hurdle technology, which relies on optimal combination of several preservation factors or hurdles. So far, more than 60 potential hurdles for food, which improves the stability and /or quality of products have been reported. In industrialized countries, the hurdle technology approach is currently of much interest for minimally processed food, which are mildly heated or fermented, and for underpinning the microbial stability and safety of foods coming from healthful foods requiring minimal packaging. In developing countries, the application of hurdle technology for foods that remain stable, safe and tasty if stored without refrigeration is of paramount importance. Much interest in intentional hurdle technology is also emerging for meat product in China and for dairy products in India.

For each stable and safe food, a certain set of hurdles is inherent, which differs in quality and intensity depending on particular product. In any case, the hurdles must keep the normal population of microorganisms in food under control. The initial population of microorganisms present in meat should not be able to leap over the

hurdles present during storage of meat; otherwise, the meat will spoil and even cause food poisoning

The multi-target approach of food preservation involving the additive and/or synergistic effects of hurdles has prompted this study on development of two different hurdle-processed buffalo meat products namely pickles and powder. The study involves the effects of spices, condiments, acetic acid, sodium nitrite and potassium sorbate as hurdles in combination with pH and moisture content on pickling, powdering, packaging, quality attributes and stability of these two buffalo meat products.

The specific objectives of the present investigation were as listed below:

* Development of convenience buffalo meat products namely pickles and powder using a combination of selected hurdles viz. pH, moisture content, chemical preservatives, spices/condiments and packaging materials.
* Evaluation of microbiological, physico-chemical, textural and organoleptic characteristics of developed buffalo meat products.
* Evaluation of shelf stability of the developed products and changes in their characteristics, especially microbial characteristics during storage at ambient temperature in different packaging materials.

Keeping in view the above objectives, the effects of pickling medium and preservatives, materials of packaging, methods of packaging (in case of meat powder only) and period of storage were examined on microbiological, physico-chemical, textural and organoleptic characteristics of developed products and their shelf stability.

Pre-rigor meat consisting of round portions comprising mostly of semi-membranous, semi-tendenous bicep femoris and quadriceps muscles of male carcass of buffalo, slaughtered by 'Halal' method was used in the study. The animal was

slaughtered 2h before the sample collection. The meat samples were packed in HDPE bags and brought to laboratory within 10 minutes after separation from carcass and immediately stored in a deep freezer at 4 ± 1°C. The bicep was trimmed off and meat samples were cut into chunks of 2.5 to 3.5cm size before conducting further studies .

Although, the weight, sex, source, method of slaughtering and collection of different meat samples, procured on different days during experiments were tried to be kept same. Anticipating qualitative changes in meat, its proximate composition was experimentally determined. The average values for pH, moisture, protein, fat and ash contents and thiobarbituric acid (TBA) of fresh meat were determined at 5.89, 75%, 19.32%, 10.29%, 1.0% and 0.3 mg/kg, respectively.

For development of meat pickles, the similar recipe as recommended for preparation of vegetable pickles was used. The untreated control as well as the meat pickles treated with 2% each of cinnamon, clove, turmeric, garlic, 0.025 % of potassium sorbate and 0.02 % sodium nitrite were developed in soybean oil medium. However, the mustard (2%) and acetic acid (6 %) treated meat pickles were developed in their own mediums.

The various microbiological characteristics viz. total plate count (TPC), coliform count, lipolytic count, acidophilic count and yeast and mold counts, physico-chemical characteristics (pH, moisture content, protein, ash content and thiobarbituric acid (TBA) values), textural characteristics (hardness, cohesiveness and gumminess) and organoleptic characteristics (colour, odour, texture, taste and palatability) of both the fresh and preserved meat pickles were determined following standard laboratory methods. Organoleptic evaluations of both products were carried out with the help of a six member panel of semi-trained panelists drawn from the laboratory/department using a nine point hedonic scale.

For storage and shelf life stability studies, the samples of all pickles developed in various mediums and treated with spices, acetic acid, potassium sorbate and sodium nitrite, were preserved in HDPE and glass jars by atmospheric packaging method. The packed samples were stored for 120 days at ambient conditions during March to September when the temperature ranged between 30 to 35 °C.

The data of all experimental studies were statistically analysed for variations among the mean values of each individual parameters. The two ways ANOVA for each quality parameter was carried out to find out any significant difference between the mean values.

For development of another hurdle processed meat product, "the meat powder", raw buffalo meat was dehydrated and transformed into powder form. Dehydration of meat was carried out in a tray drier at 180°C to 60°C for 3 days. The dehydrated meat pieces were powdered in a grinder. For stability studies, the developed powder samples were packed in two different packaging materials namely combination film and autoclavable polythene. In this study, the hurdle parameters were incorporated also in the form of treatments. To increase the shelf life, the meat after dehydration was treated with clove, turmeric and potassium sorbate. Three other treatments used were related to packaging. All the samples were stored at ambient temperature during August to February when the atmospheric temperature ranged between 35°C to 12°C. The various microbiological, physico-chemical and sensory characteristics of preserved meat powder samples were evaluated by using standard techniques. To test the functional suitability of preserved meat powder, the powder was reconstituted and used for developing Shami-kababs. The sensory characteristics of developed kababs from preserved meat powder samples were also evaluated. All the data were statistically analyzed. The results are summarized as under:

5.1. Product one: Hurdle processed meat pickles

* Treatment of meat with certain natural products (spices and condiments) and synthetic chemicals as hurdles parameters during pickling resulted in differential reduction in pH in a time-dependent manner during 120 days of storage at ambient temperature. Spices used as natural preservatives, particularly the cinnamon, clove and garlic significantly reduced the pH values of pickles with in 20 days of storage vis-à-vis untreated control and synthetic preservatives, except acetic acid. Treatments with the various preservatives resisted the change in pH of meat pickle in the order as potassium sorbate > sodium nitrite > mustard > turmeric > garlic > clove > cinnamon > acetic acid. The low pH in acetic acid, cinnamon, clove and garlic treated meat pickles prevented the spoilage and extended the shelf life of the product by retarding microbial growth.
* Pickling treatments with natural products as preservatives have added to the protein content of meat as compared to control. The protein values of meat pickles increased in the order as clove > turmeric > cinnamon > garlic > mustard. The protein content of treated pickle remained unchanged upon storage, however, the decrease in protein content in control meat pickle was noticed during 120 days of storage. The results suggested the beneficial effects of spices and condiments on stability of meat pickles.
* Pickling treatments with natural and synthetic preservatives maintained the TBA number of meat during storage, however, it increased substantially as a function of time in case of control during storage at ambient temperature. Slight increase in TBA values was noticed in garlic, mustard, potassium sorbate, and sodium nitrite treated pickles. Nevertheless, the TBA values invariably lie with in the threshold limit of 1mg/kg at which lipid rancidity occurs. Comparative analysis revealed that the meat pickles packed in glass jars exhibit lower TBA values than the HDPE jars.

- The ash contents both in control and treated meat pickles with spices / condiments were found to increase significantly as compared to raw meat and did not changed with storage. Insignificant effects of storage period and packaging material on ash content of pickles were observed.
- The effective concentrations of spices/condiment and synthetic chemicals used in the study were determined based on their MIC value against the pure cultures of common bacteria associated with meat spoilage. The MIC of the extracts of spices/condiments against *Staphylococcus aureus, Salmonella enteritidis, E.coli* and *Listeria monocytogenes* were determined to be 2%. However, the MIC of synthetic preservatives were determined to be 0.02%, 0.025% and 6% for sodium nitrite, potassium sorbate and acetic acid, respectively. Both the natural and synthetic products at these sub-lethal concentrations were applied as treatments to meat pickles.
- Pickling medium, preservatives, storage condition and time period have significantly influenced the microbial quality of meat pickles. Nevertheless, in all cases the pickles were in edible condition (hedonic rating 5.67 to 8.67) even after 120 days storage at ambient temperatures. Significant reduction in microbial population (TPC) was observed as a function of storage time in all treatments irrespective of packaging material. On the contrary, the TPC increased in untreated control and potassium sorbate treated meat pickles in both packaging materials.
- The efficacy of natural products (spices/condiments) for microbial control was observed to be in the order as clove > garlic > turmeric > cinnamon > mustard. Amongst the synthetic preservatives, the efficacy was in the order as sodium nitrite > acetic acid > potassium sorbate. Packaging of pickles in glass jar was found to be safer than HDPE jar for long term storage under ambient conditions.
- Treatment of meat with both the natural and synthetic preservatives significantly reduced the coliform counts in pickles. However, pickles treated with mustard and

potassium sorbate exhibited recurrence of *E.coli* growth after 20 days of storage at ambient temperature, which increased further with increase in storage period. Glass jar was found to be a better packaging material for long term storage of meat pickles. Cinnamon, clove, turmeric and garlic treatments exhibited effective anti-microbial activity, and suppressed the growth of coliforms during long term storage.

* Treatments with the natural products as preservatives resulted in significant decrease in *Staphylococcus* count in meat pickles after 20^{th} days of storage. Amongst synthetic preservatives sodium nitrite and acetic acid suppressed the growth of *Staphylococcus* group of bacteria. However, potassium sorbate treatment increased the count during storage. Glass jar was suggested to be better a packaging material than HDPE jar.
* Treatment of meat pickles with clove, turmeric, cinnamon, effectively also checked the growth of proteolytic microorganisms. Moreover, all five natural products at 2% level viz. cinnamon, clove, turmeric, garlic and mustard inhibited the growth of lipolytic microorganisms. The synthetic chemicals viz. sodium nitrite, potassium sorbate and acetic acid also caused 100% inhibition of the growth of lipolytic bacteria up to 20 to 40 days. The lipolytic bacteria, however, reappeared upon prolonged storage at room temperature.
* Treatments of meat pickles with cinnamon, clove, turmeric, acetic acid and potassium sorbate were effective in controlling the growth of yeast and mold counts during 120 days storage. However, garlic in meat pickles was not effective in checking the growth of yeast and molds count.
* The pickles treated with all natural and synthetic preservatives resulted in the products, which were placed in 'liked very much' category based on sensory evaluation, as far as the colour is concerned. The sensory score, however, decreased during storage. Glass jar proved better than HDPE jar in retaining the colour of pickles during storage.

- The odour scores of pickles increased significantly with storage period. All pickles except acetic acid treated pickles were rated as 'liked very much' on odour scores. Mustard and clove treated pickles were most liked while turmeric and acetic acid treated pickles were least liked. From odour score point of view also glass jar was better than HDPE jar for long term storage of pickles.
- Invariably all the treatments significantly influenced the texture of pickles during storage at ambient temperature. The storage significantly improved the texture of all pickles. On the 120^{th} day (at the end of storage) cinnamon, turmeric and mustard treated pickles had the highest score for texture which was liked very much. Acetic acid pickles, however, were slightly disliked. No significant effect of packaging material on textural qualities of meat pickles was observed. Treatment with clove and cinnamon and acetic acids significantly reduced the hardness of the pickles.

5.2. Product two: hurdle processed meat powder

- The meat powder had average particle size of 0.26mm, moisture content of 4.0 to 4.2%, pH of 5.39 to 6.03, protein content of 67.1%, fat content of 11.77% and TBA number of 0.29 ± 0.07mg/kg and exhibited a shelf life of 120 days.
- The moisture content, pH, fat content and ash content of meat powder did not significantly changed during storage upto 120 days at ambient temperature. The treatment of meat before drying and powdering had insignificant effect on pH and protein content of meat powder.
- The two packaging materials used for meat powder namely combination film and autoclavable polyethylene also had insignificant effect on pH and protein content of meat powder.
- Meat dehydration and powdering significantly increased the fat content of meat product. Treatment of meat powder with different preservatives after dehydration had

insignificant effects on fat content of powder as was the case with two packaging materials used for storage of meat powder.

- Treatments of meat after dehydration and powdering significantly increased the TBA number of meat powder during ambient storage, to extent that the product crossed the threshold level of rancidity, probably due to degradation of fatty acid.
- Meat powder treatments with clove and turmeric after dehydration and subsequent powdering completely checked the TPC of meat powder. The powder remained microbe free virtually with no detectable *Staphylococcus*, proteolytic or lipolytic bacteria throughout 120 days storage period. The bacterial count, however, increased in untreated control meat powder. Autoclavable polyethylene as packaging material in comparison to combination film was observed as inappropriate packaging material. Vacuum packaging was assessed to be better packaging alternative for maintaining low microbial count vis-à-vis modified atmosphere packaging of meat powder.
- Yeast and mold counts in untreated control meat powder have significantly increased during storage for 120 days. However, treatment with clove effectively controlled the growth of these microorganisms. Turmeric treated meat powder exhibited some yeast and mold growth after 60 days storage in the powder packed in autoclavable polythene. Potassium sorbate completely prevented the growth of yeast and mold in both packaging materials. As compared to CO_2 and N_2 flushing methods, the bacterial and fungal growth control was very good in vacuum packed meat powder.
- The developed meat powder was rated as 'liked very much' with respect to colour and odour. Treatments of meat significantly improved organoleptic characteristics. Vacuum packaging was the best method as compared to other methods of packaging.
- The Kababs developed from meat powder preserved for 120 days were rated as 'most liked' because of good organoleptic scores.

- To the best of author's understanding, the present investigation is the first effort in global scenario for preparation, preservation and utilization of buffalo meat in powdered form. Such a product could be stored safely at ambient temperature for 120 days using spices/condiments as preservatives, maintaining the low TBA value and microbial counts. Also, this unique methods of meat preservation exhibits enormous potential of utilization of meat for a long duration and faster reconstitution to produce variety of meat products viz. kababs, patties and balls of varying dimension.

5.3. Conclusions

Based on above studies following conclusions are drawn:

Pickling of fresh buffalo meat and drying–cum–powdering of buffalo meat, using selected hurdle parameters viz pH, preservatives, dehydration, and packaging, etc. proved to be effective methods for controlling the meat microflora and preserving the meat for subsequent long term consumption.

The pickling medium (soybean/mustard oil), spices, organic acids and salts, packaging material and methods, storage temperatures, etc. create a distinctive micro-environment in and around the meat products, which inhibits the proliferation of the deleterious microflora responsible for meat spoilage. Pickling treatments and subsequent fermentation contribute to improvement in flavour and textural properties. The primary fermentation product lowers the meat pH and contributes to the stability of these pickles against food borne pathogens and other less desirable spoilage microorganisms.

Among the spices used in present study, the role of garlic, turmeric and clove in management of diabetes mellitus and lipid metabolism, and as anti–inflammatory agent, respectively are well documented. Development of meat pickle with these spices adds medicinal value to product as a bonus. These spices are also known to stimulate pancreatic digestive enzymes such as lipase, amylase and protease, which

may play a crucial role in digestion and reduction in food transit time in gastrointestinal tract.

Although, the spices/condiments used in present study were initially selected as hurdle parameters owing to their antimicrobial activity, to extend the shelf life of products (pickles and powder), however, the correlation of data with inherent characteristics of spices outspread their efficacy. The spices/condiments used in preparation/preservation of meat pickles and powder could exhibit multi-pronged benefits as (i) antimicrobial preservative, (ii) flavouring agent (iii) natural therapeutic agent with the host of beneficial physiological effects. Keeping in view many promising health beneficial effects, such food adjuncts could be regarded as 'neutraceuticals', which not only makes the food spicy but more healthy too.

Thus, both the hurdle processed meat products namely pickles and powder have the advantage of a longer shelf life (120 days), desirable organoleptic attributes, safe ingredients and low cost of processing. The longer shelf life of both the products at ambient temperature and good nutritive/medicinal values may add great convenience to many meat consumers. Both the products could also serve as protein supplement for the defense establishments, hotels/ restaurants/ fast food shops, and travelers etc. due to the logical advantages of 'easy to pack', 'easy to cook' and 'ready to eat' properties.

Recommendations

Based on the results of present investigation following recommendations are made:-

1. Hurdle technology offers tremendous potential for preservation of variety of meat products. The present investigation has considered only a combination of selected hurdle parameters for preservation of meat pickles and meat powder. Impact of several other hurdle parameters should be assessed on similar and/or different meat products.
2. In the present study, the effect of various methods of packaging viz. vacuum packaging, modified atmosphere packaging and atmosphere packaging were investigated only in case of meat powder. Such studies need to be extended for meat pickles and other intermediate moisture foods products of meat. Also effects of other methods viz controlled atmosphere packaging, aseptic packaging materials for such purposes need to be examined.
3. Commercial exploitation of the results of present investigation and its economical viablity also need to be worked out.
4. Keeping in view, the scope of asceptic packaging conditions, other preservatives in combination with other hurdle parameters may lead to development of a variety of convenience, ready to eat /serve and highly stable meat products. Studies on these aspects also need to be carried out. It is possible that a range of healthy meat based 'Nutraceuticals' may be developed by fortifying meat with selected food additives.

Annexure

Table A.1 ANOVA analysis of pH values of buffalo meat pickles during storage of 120 days at ambient temperature.

		P_1				P_2			
Source	**df**	**ss**	**mss**	**F-cal**	**F-Table 0.05**	**ss**	**mss**	**F-cal**	**F-Table 0.05**
Replications	2	01.763	0.882			01.420	0.710		
Treatments	8	33.576	4.197	47.702	2.02	36.747	4.593	63.431	2.02
Storage	6	10.772	1.795	20.406	2.18	06.296	1.049	14.490	2.18
Interaction	48	08.048	0.168	01.906	1.48	06.847	0.143	01.970	1.48
Error	124	10.910	0.088			08.979	0.072		
Total	188	65.070				60.290			

Table A.2 ANOVA of protein content of buffalo meat pickles during storage of 120 days at ambient temperature

		P_1				P_2			
Source	**df**	**ss**	**mss**	**F-cal**	**F-Table 0.05**	**ss**	**mss**	**F-cal**	**F-Table 0.05**
Replications	2	005.363	02.681			004.391	02.195		
Treatments	8	155.526	19.441	99.976	2.02	136.033	17.004	75.970	2.02
Storage	6	020.426	03.404	17.507	2.18	018.252	3.0419	13.591	2.18
Interaction	48	086.142	01.795	09.229	1.48	086.621	01.806	08.062	1.48
Error	124	024.112	00.194			027.755	00.224		
Total	188	291.569				273.051			

P_1 =HDPE jar, P_2 =Glass jar

Table A.3 ANOVA of TBA value of buffalo meat pickles during storage of 120 days at ambient temperature

		P_1				P_2			
Source	**df**	**ss**	**mss**	**F-cal**	**F-Table 0.05**	**ss**	**mss**	**F-cal**	**F-Table 0.05**
Replications	2	0.003	0.001			0.000	6.560		
Treatments	8	1.768	0.221	108.760	2.02	1.207	0.151	83.369	2.02
Storage	6	0.338	0.056	027.751	2.18	0.188	0.031	17.361	2.18
Interaction	48	0.884	0.018	009.060	1.48	0.554	0.011	06.377	1.48
Error	124	0.252	0.002			0.224	0.002		
Total	188	3.245				2.173			

Table A. 4 ANOVA of ash content of buffalo meat pickles during storage of 120 days at ambient temperature

		P_1				P_2			
Source	**df**	**ss**	**mss**	**F-cal**	**F-Table 0.05**	**ss**	**mss**	**F-cal**	**F-Table 0.05**
Replications	2	0.233	0.117			0.240	0.120		
Treatments	8	0.363	0.045	9.175	2.97	0.359	0.045	8.837	2.97
Storage	6	0.002	0.000	0.069	3.70	0.004	0.001	0.146	3.70
Interaction	48	0.017	0.000	0.070	1.51	0.021	0.000	0.085	1.51
Error	124	0.614	0.005			0.630	0.005		
Total	188	1.229				1.255			

P_1 =HDPE jar, P_2 =Glass jar

Table A.5 ANOVA of TPC counts of buffalo meat pickles during storage of 120 days at ambient temperature

		P_1				P_2			
Source	**df**	**ss**	**mss**	**F-cal**	**F-Table 0.05**	**ss**	**mss**	**F-cal**	**F-Table 0.05**
Replicates	2	3.152	1.576			1.381	0.691		
Treatments	8	763.401	95.425	389.223	2.02	694.832	86.854	523.860	2.02
Storage	6	53.742	8.957	36.534	2.18	74.818	12.470	75.210	2.18
Interaction	48	269.678	5.618	22.916	1.48	240.720	5.015	30.248	1.48
Error	124	30.401	0.245			20.559	0.166		
Total	188	1120.38				1032.310			

Table A.6 ANOVA of coliforms counts of buffalo meat pickles during storage of 120 days at ambient temperature

		P_1				P_2			
Source	**df**	**ss**	**mss**	**F-cal**	**F-Table 0.05**	**ss**	**mss**	**F-cal**	**F-Table 0.05**
Replicates	2	0.097	0.048	1.099		0.003	0.001	0.020	
Treatments	8	219.454	27.432	621.884	2.02	140.783	17.598	240.401	2.02
Storage	6	15.517	2.586	58.630	2.18	5.709	0.951	12.999	2.18
Interaction	48	72.563	1.512	34.271	1.48	49.716	1.0358	14.149	1.48
Error	124	5.470	0.044			9.077	0.073		
Total	188	313.101				205.289			

P_1 =HDPE jar, **P_2** =Glass jar

Table A.7 ANOVA of *Staphylococcal* counts of buffalo meat pickles during storage of 120 days at ambient temperature

		P_1				P_2		
Source	**df**	**ss**	**mss**	**F-cal**	**F-Table 0.05**	**ss**	**mss**	**F-cal**	**F-Table 0.05**
Replicates	2	0.035	0.018			000.008	00.004		
Treatments	8	111.600	13.95	186.168	2.02	081.279	10.160	118.346	2.02
Storage	6	8.357	1.393	018.589	2.18	011.784	01.964	022.878	2.18
Interaction	48	45.066	0.939	012.530	1.48	043.062	00.897	010.450	1.48
Error	124	9.292	0.075			010.645	00.086		
Total	188	174.350				146.779			

Table A.8 ANOVA of proteolytic counts of buffalo meat pickles during storage of 120 days at ambient temperature

		P_1				**P_2**		
Source	**df**	**ss**	**mss**	**F-cal**	**F-Table 0.05**	**ss**	**mss**	**F-cal**	**F-Table 0.05**
Replicates	2	0.617	0.308			0.320	0.160		
Treatments	8	67.816	8.477	121.240	2.02	72.621	9.078	164.393	2.02
Storage	6	7.808	1.302	18.612	2.18	4.872	0.812	14.706	2.18
Interaction	48	28.184	0.587	8.398	1.48	22.623	0.471	8.535	1.48
Error	124	8.670	0.070			6.847	0.055		
Total	188	113.095				107.283			

P_1 =HDPE jar, **P_2** =Glass jar

Table A.9 of ANOVA of lipoytic counts of buffalo meat pickles during storage of 120 days at ambient temperature

		P_1				P_2			
Source	**df**	**ss**	**mss**	**F-cal**	**F-Table 0.05**	**ss**	**mss**	**F-cal**	**F-Table 0.05**
Replicates	2	0.100	0.050	2.142		0.198	0.099	2.604	
Treatments	8	85.699	10.712	457.063	2.02	39.176	4.897	128.781	2.02
Storage	6	21.546	3.591	153.216	2.18	10.826	1.804	47.449	2.18
Interaction	48	34.764	0.724	30.902	1.48	18.647	0.388	10.216	1.48
Error	124	2.906	0.023			4.715	0.0380		
Total	188	145.016				73.563			

Table A.10 ANOVA of Y&M counts of buffalo meat pickles during storage of 120 days at ambient temperature.

		P_1				P_2			
Source	**df**	**ss**	**mss**	**F-cal**	**F-Table 0.05**	**ss**	**mss**	**F-cal**	**F-Table 0.05**
Replicates	2	000.802	000.401			0000.666	000.333	002.349	
Treatments	8	1011.51	126.439	226.182	2.02	0841.014	105.127	741.754	2.02
Storage	6	024.617	004.103	007.339	2.18	0028.761	004.793	033.822	2.18
Interaction	48	268.399	005.592	010.003	1.48	0228.527	004.761	033.593	1.48
Error	124	069.318	000.559			0017.574	000.142		
Total	188	1374.647				1116.541			

P_1 =HDPE jar, P_2 =Glass jar

Table A.11 ANOVA of colour counts of buffalo meat pickles during storage of 120 days at ambient temperature

		P_1				P_2			
Source	**df**	**ss**	**mss**	**F-cal**	**F-Table 0.05**	**ss**	**mss**	**F-cal**	**F-Table 0.05**
Replicates	2	12.603	6.306			13.841	6.921		
Treatments	8	46.952	5.869	7.576	2.02	33.048	4.131	4.594	2.02
Storage	6	7.175	1.196	1.544	2.18	5.259	0.877	0.975	2.18
Interaction	48	17.492	0.364	0.470	1.48	14.360	0.299	0.333	1.48
Error	124	96.063	0.775			111.492	0.899		
Total	188	180.286				178.000			

Table A.12 ANOVA of odour buffalo meat pickles during storage of 120 days at ambient temperature

		P_1				P_2			
Source	**df**	**ss**	**mss**	**F-cal**	**F-Table 0.05**	**ss**	**mss**	**F-cal**	**F-Table 0.05**
Replicates	2	10.889	5.444			1.725	0.862	0.930	
Treatments	8	290.857	36.357	35.282	2.02	307.249	38.406	41.433	2.02
Storage	6	21.291	3.549	3.444	2.18	1.767	0.295	0.318	2.18
Interaction	48	30.995	0.646	0.627	1.48	34.900	0.727	0.784	1.48
Error	124	127.778	1.030			114.942	0.928		
Total	188	481.810				460.582			

P_1 =HDPE jar, P_2 =Glass jar

Table A.13 ANOVA of texture counts of buffalo meat pickles during storage of 120 days at ambient temperature

		P_1				P_2			
Source	**df**	**ss**	**mss**	**F-cal**	**F-Table 0.05**	**ss**	**mss**	**F-cal**	**F-Table 0.05**
Replicates	2	14.106	7.053			12.519	6.259		
Treatments	8	42.106	5.263	8.169	2.02	45.439	5.680	5.735	2.02
Storage	6	168.995	28.166	43.715	2.18	177.661	29.610	29.896	2.18
Interaction	48	39.672	0.826	1.283	1.48	35.005	0.729	0.736	1.48
Error	124	79.894	0.644			122.815	0.990		
Total	188	344.773				393.439			

Table A.14 ANOVA of taste counts of buffalo meat pickles during storage of 120 days at ambient temperature

		P_1				P_2			
Source	**df**	**ss**	**mss**	**F-cal**	**F-Table 0.05**	**ss**	**mss**	**F-cal**	**F-Table 0.05**
Replicates	2	16.519	8.259			25.175	12.587		
Treatments	8	441.598	55.200	51.536	2.02	439.429	54.929	49.063	2.02
Storage	6	3.365	0.561	0.524	2.18	3.534	0.589	0.526	2.18
Interaction	48	70.921	1.478	1.379	1.48	70.275	1.464	1.308	1.48
Error	124	132.815	1.071			138.825	1.120		
Total	188	665.217				677.238			

P_1 =HDPE jar, P_2 =Glass jar

Table A.15 ANOVA of palatability counts of buffalo meat pickles during storage of 120 days at ambient temperature

		P_1				P_2			
Source	**df**	**ss**	**mss**	**F-cal**	**F-Table 0.05**	**ss**	**mss**	**F-cal**	**F-Table 0.05**
Replicates	2	43.556	21.778			43.249	21.624		
Treatments	8	234.191	29.274	30.306	2.02	241.407	30.176	28.046	2.02
Storage	6	10.497	1.750	1.811	2.18	11.333	1.889	1.756	2.18
Interaction	48	41.217	0.859	0.889	1.48	39.333	0.819	0.762	1.48
Error	124	119.778	0.966			133.418	1.076		
Total	188	449.238				468.741			

Table A.16 ANOVA of moisture content analysis of meat powder during storage of 120 days at ambient temperature

		P_3				P_4			
Source	**df**	**ss**	**mss**	**F-cal**	**F-Table 0.05**	**ss**	**mss**	**F-cal**	**F-Table 0.05**
Replications	2	1.419	0.710			0.543	0.271		
Treatments	6	0.263	0.044	01.482	3.71	0.352	0.059	2.482	3.71
Storage	6	0.019	0.003	00.106	3.71	0.037	0.006	0.261	3.71
Interaction	36	0.042	0.001	00.040	1.63	0.125	0.003	0.147	1.63
Error	96	2.834	0.030			2.270	0.024		
Total	146	4.577				3.327			

P_1 =HDPE jar, P_2 =Glass jar, P_3 =Combination film, P_4 = autoclavable polythene

Table A.17 ANOVA of pH of buffalo meat powder during storage of 120 days at ambient temperature

		P_3				P_4			
Source	**df**	**ss**	**mss**	**F-cal**	**F-Table 0.05**	**ss**	**mss**	**F-cal**	**F-Table 0.05**
Replications	2	0.092	0.046			0.153	0.076		
Treatments	6	0.477	0.080	4.059	3.71	0.502	0.084	4.854	3.71
Storage	6	0.030	0.005	0.251	3.71	0.041	0.007	0.395	3.71
Interaction	36	0.066	0.002	0.095	1.63	0.078	0.002	0.126	1.63
Error	96	1.881	0.020			1.654	0.017		
Total	146	2.547				2.427			

Table A.18 ANOVA of protein of buffalo meat powder during storage of 120 days at ambient temperature

		P_3				P_4			
Source	**df**	**ss**	**mss**	**F-cal**	**F-Table 0.05**	**ss**	**mss**	**F-cal**	**F-Table 0.05**
Replications	2	13.782	6.891			15.707	7.853		
Treatments	6	01.686	0.281	3.598	3.71	02.156	0.359	4.733	3.71
Storage	6	00.213	0.036	0.455	3.71	00.216	0.036	0.474	3.71
Interaction	36	00.500	0.014	0.178	1.63	00.331	0.009	0.121	1.63
Error	96	07.498	0.078			07.287	0.076		
Total	146	23.680				25.696			

P_3 =Combination film, **P_4** = autoclavable polythene

Table A.19 ANOVA of fat of buffalo meat powder during storage of 120 days at ambient temperature

		P_3				P_4			
Source	**df**	**ss**	**mss**	**F-cal**	**F-Table 0.05**	**ss**	**mss**	**F-cal**	**F-Table 0.05**
Replications	2	0.198	0.099			0.110	0.055		
Treatments	6	2.763	0.460	11.940	3.71	3.090	0.514	15.979	3.71
Storage	6	0.121	0.020	0.522	3.71	0.157	0.026	0.813	3.71
Interaction	36	0.298	0.008	0.215	1.63	0.337	0.009	0.291	1.63
Error	96	3.702	0.039			3.091	0.032		
Total	146	7.082				6.781			

Table A.20 ANOVA of TBA of buffalo meat powder during storage of 120 days at ambient temperature

		P_3				P_4			
Source	**df**	**ss**	**mss**	**F-cal**	**F-Table 0.05**	**ss**	**mss**	**F-cal**	**F-Table 0.05**
Replications	2	0.014	0.007			0.035	0.018		
Treatments	6	2.254	0.376	36.590	3.71	2.915	0.486	50.897	3.71
Storage	6	1.676	0.279	27.203	3.71	2.478	0.413	43.276	3.71
Interaction	36	1.746	0.048	04.723	1.63	1.898	0.053	05.524	1.63
Error	96	0.986	0.010			0.916	0.009		
Total	146	6.676				8.243			

P_3 =Combination film, P_4 = autoclavable polythene

Table A.21 ANOVA of ash content of buffalo meat powder during storage of 120 days at ambient temperature

		P_3				P_4			
Source	**df**	**ss**	**mss**	**F-cal**	**F-Table 0.05**	**ss**	**mss**	**F-cal**	**F-Table 0.05**
Replications	2	1.78	0.888	144.21		1.757	0.879	138.417	
Treatments	6	0.0013	0.00023	0.037	3.71	0.0019	0.0003	0.05001	3.71
Storage	6	0.021	0.0036	0.578	3.71	0.02	0.003	0.525	3.71
Interaction	36	0.0177	0.0005	0.080	1.63	0.018	0.0005	0.079	1.63
Error	96	0.591	0.006			0.609	0.006		
Total	146	2.407				2.407			

Table A.22 Table of ANOVA of TPC of buffalo meat powder during storage of 120 days at ambient temperature

		P_3				P_4			
Source	**df**	**ss**	**mss**	**F-cal**	**F-Table 0.05**	**ss**	**mss**	**F-cal**	**F-Table 0.05**
Replications	2	00.015	0.008			000.011	00.005		
Treatments	6	43.994	7.332	1775.728	3.71	081.343	13.557	312.239	3.71
Storage	6	03.431	0.572	0138.500	3.71	063.297	10.550	242.969	3.71
Interaction	36	11.268	0.313	0075.799	1.63	105.887	02.941	67.742	1.63
Error	96	00.396	0.004			004.168	00.043		
Total	146	59.105				254.706			

P_3 =Combination film, P_4 = autoclavable polythene

Table A.23 ANOVA of *Staphylococcus* of buffalo meat powder during storage of 120 days at ambient temperature

		P_3				P_4			
Source	**df**	**ss**	**mss**	**F-cal**	**F-Table 0.05**	**ss**	**mss**	**F-cal**	**F-Table 0.05**
Replications	2	0.067	0.034			0.007	0.003		
Treatments	6	105.291	17.548	1355.028	3.71	226.472	37.745	5387.903	3.71
Storage	6	10.111	1.685	130.119	3.71	20.405	3.401	485.447	3.71
Interaction	36	60.664	1.685	130.119	1.63	104.756	2.910	415.365	1.63
Error	96	1.243	0.013			0.673	0.007		
Total	146	177.376				352.312			

Table A.24 ANOVA of proteolytic of buffalo meat powder during storage of 120 days at ambient temperature

		P_3				P_4			
Source	**df**	**ss**	**mss**	**F-cal**	**F-Table 0.05**	**ss**	**mss**	**F-cal**	**F-Table 0.05**
Replications	2	000.011	00.006			000.009	00.004		
Treatments	6	137.638	22.940	3917.46	3.71	122.746	20.458	15235.550	3.71
Storage	6	005.914	00.986	168.320	3.71	022.912	03.819	02843.849	3.71
Interaction	36	035.483	00.986	168.320	1.63	064.136	01.782	01326.786	1.63
Error	96	000.562	00.006			000.129	00.001		
Total	146	179.608				209.931			

P_3 =Combination film, P_4 = autoclavable polythene

Table A.25 ANOVA of Y&M of buffalo meat powder during storage of 120 days at ambient temperature

		P_3				P_4			
Source	**df**	**ss**	**mss**	**F-cal**	**F-Table 0.05**	**ss**	**mss**	**F-cal**	**F-Table 0.05**
Replications	2	000.013	00.006			000.008	00.004		
Treatments	6	506.838	84.473	1681.849	3.71	551.778	91.963	2387.785	3.71
Storage	6	048.081	08.013	0159.547	3.71	157.074	26.179	0679.726	3.71
Interaction	36	223.733	06.215	0123.736	1.63	261.887	07.275	0188.883	1.63
Error	96	004.822	00.050			003.697	00.039		
Total	146	783.487				974.443			

Table A.26 ANOVA of colour of buffalo meat powder during storage of 120 days at ambient temperature

		P_1				P_2			
Source	**df**	**ss**	**mss**	**F-cal**	**F-Table 0.05**	**ss**	**mss**	**F-cal**	**F-Table 0.05**
Replicates	2	12.603	6.306			13.841	6.921		
Treatments	8	46.952	5.869	7.576	2.02	33.048	4.131	4.594	2.02
Storage	6	7.175	1.196	1.544	2.18	5.259	0.877	0.975	2.18
Interaction	48	17.492	0.364	0.470	1.48	14.360	0.299	0.333	1.48
Error	124	96.063	0.775			111.492	0.899		
Total	188	180.286				178.000			

P_3 =Combination film, P_4 = autoclavable polythene

Table A.27 ANOVA of odour of buffalo meat powder during storage of 120 days at ambient temperature

		P_3				P_4			
Source	**df**	**ss**	**mss**	**F-cal**	**F-Table 0.05**	**ss**	**mss**	**F-cal**	**F-Table 0.05**
Replications	2	2.340	1.170			0.286	0.143		
Treatments	6	24.925	4.154	9.091	2.20	26.803	4.467	11.910	2.20
Storage	6	3.496	0.583	1.275	2.20	15.279	2.546	6.789	2.20
Interaction	36	5.741	0.159	0.349	1.55	10.150	0.282	0.752	1.55
Error	96	44.326	0.457			36.380	0.375		
Total	146	80.829				88.898			

P_3 =Combination film, P_4 = autoclavable polythene

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